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(71) Applicant: CHILDREN'S HOSPITAL OAKLAND RESEARCH INSTITUTE [US/US]; 747 Fifty Second Street, Oakland, CA 96409–1809 (US).

(72) Inventors: SABA, Julie, D.; Apartment 7317, 6401 Bay Street, Emeryville, CA 95608 (US). ZHOU, Jianhui; 246 Bearritz Court, Redwood City, CA 94065 (US).

(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

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(57) Abstract

Compositions, methods and kits for diagnosing and treating cancer are provided. Therapeutic compositions may comprise agents that modulate the expression or activity of a sphingosine–1–phosphate lyase (SPL). Such compositions may be administered to a mammal afflicted with cancer. Diagnostic methods and kits may employ an agent suitable for detecting alterations in endogenous SPL. Such methods and kits may be used to detect the presence of a cancer or to evaluate the prognosis of a known disease. SPL polypeptides, polynucleotides and antibodies are also provided.

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GGT GAT GAT TTG ATC CAC TTA CAA ACA ATC GCA TAC GAA AAA TAT TGC   576
Gly Asp Asp Leu Ile His Leu Gln Thr Ile Ala Tyr Glu Lys Tyr Cys
            180                 185                 190

GTT GCC AAT CAA TTA CAT CCC GAT GTC TTT CCT GCC GTA CGT AAA ATG   624
Val Ala Asn Gln Leu His Pro Asp Val Phe Pro Ala Val Arg Lys Met
        195                 200                 205

GAA TCC GAA GTG GTT TCT ATG GTT TTA AGA ATG TTT AAT GCC CCT TCT   672
Glu Ser Glu Val Val Ser Met Val Leu Arg Met Phe Asn Ala Pro Ser
    210                 215                 220

GAT ACA GGT TGT GGT ACC ACA ACT TCA GGT GGT ACA GAA TCC TTG CTT   720
Asp Thr Gly Cys Gly Thr Thr Thr Ser Gly Gly Thr Glu Ser Leu Leu
225                 230                 235                 240

TTA GCA TGT CTG AGC GCT AAA ATG TAT GCC CTT CAT CAT CGT GGA ATC   768
Leu Ala Cys Leu Ser Ala Lys Met Tyr Ala Leu His His Arg Gly Ile
                245                 250                 255

ACC GAA CCA GAA ATA ATT GCT CCC GTA ACT GCA CAT GCT GGG TTT GAC   816
Thr Glu Pro Glu Ile Ile Ala Pro Val Thr Ala His Ala Gly Phe Asp
            260                 265                 270

AAA GCT GCT TAT TAC TTT GGC ATG AAG CTA CGC CAC GTG GAG CTA GAT   864
Lys Ala Ala Tyr Tyr Phe Gly Met Lys Leu Arg His Val Glu Leu Asp
        275                 280                 285

CCA ACG ACA TAT CAA GTG GAC CTG GGA AAA GTG AAA AAA TTC ATC AAT   912
Pro Thr Thr Tyr Gln Val Asp Leu Gly Lys Val Lys Lys Phe Ile Asn
    290                 295                 300

AAG AAC ACA ATT TTA CTG GTC GGT TCC GCT CCA AAC TTT CCT CAT GGT   960
Lys Asn Thr Ile Leu Leu Val Gly Ser Ala Pro Asn Phe Pro His Gly
305                 310                 315                 320

ATT GCC GAT GAT ATT GAA GGA TTG GGT AAA ATA GCA CAA AAA TAT AAA  1008
Ile Ala Asp Asp Ile Glu Gly Leu Gly Lys Ile Ala Gln Lys Tyr Lys
                325                 330                 335

CTT CCT TTA CAC GTC GAC AGT TGT CTA GGT TCC TTT ATT GTT TCA TTT  1056
Leu Pro Leu His Val Asp Ser Cys Leu Gly Ser Phe Ile Val Ser Phe
            340                 345                 350
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Description

SPHINGOSINE-1-PHOSPHATE LYASE POLYPEPTIDES, POLYNUCLEOTIDES AND MODULATING AGENTS AND METHODS OF USE THEREFOR

Technical Field

The present invention relates generally to cancer detection and therapy. The invention is more particularly related to sphingosine-1-phosphate lyase polynucleotides and polypeptides, and to agents that modulate the expression and/or activity of such polypeptides. Such agents may be used, for example, to diagnose and/or treat cancers such as breast cancer.

Background of the Invention

Breast cancer is a significant health problem for women in the United States and throughout the world. Although advances have been made in detection and treatment of the disease, breast cancer remains the most common form of cancer, and the second leading cause of cancer death, in American women. Among African-American women and women between 15 and 54 years of age, breast cancer is the leading cause of cancer death. One out of every eight women in the United States will develop breast cancer, a risk which has increased 52% during 1950-1990. In 1994, it is estimated that 182,000 new cases of female breast cancer were diagnosed, and 46,000 women died from the disease.

No vaccine or other universally successful method for the prevention or treatment of breast cancer is currently available. Management of the disease currently relies on a combination of early diagnosis (through routine breast screening procedures) and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of treatment for a particular breast cancer is often selected based on a variety of prognostic parameters, including an analysis of

specific tumor markers. However, the use of established markers often leads to a result that is difficult to interpret.

With current therapies, tumor invasiveness and metastasis is a critical determinant in the outcome for breast cancer patients. Although the five year survival for women diagnosed with localized breast cancer is about 90%, the five year survival drops to 18% for women whose disease has metastasized. Present therapies are inadequate for inhibiting tumor invasiveness for the large population of women with this severe disease.

Accordingly, improvements are needed in the treatment, diagnosis and prevention of breast cancer. The present invention fulfills this need and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention provides compositions and methods for the diagnosis and therapy of cancer. Within one aspect, the present invention provides isolated polynucleotides comprising a sequence selected from the group consisting of: (a) a sequence recited in SEQ ID NO:1; (b) a sequence recited in SEQ ID NO:3; (c) nucleotide sequences that hybridize to a polynucleotide complementary to either of the foregoing sequences under moderately stringent conditions, wherein the nucleotide sequences encode polypeptides having sphingosine-1-phosphate lyase activity; and (d) nucleotide sequences that encode a polypeptide encoded by any of the foregoing sequences.

Within a related aspect, an isolated polynucleotide is provided that encodes a polypeptide recited in SEQ ID NO:2, or a variant of such a polypeptide that has sphingosine-1-phosphate lyase activity. In another related aspect, an isolated polynucleotide comprising a sequence recited in SEQ ID NO:4, or a variant of such a polypeptide that has sphingosine-1-phosphate lyase activity, is provided.

Recombinant expression vectors comprising any of the foregoing polynucleotides, and host cells transformed or transfected with such expression vectors, are also provided.

Within further aspects, SPL polypeptides are provided. Such polypeptides may be encoded by any of the foregoing polynucleotides. Alternatively, a polypeptide may comprise an amino acid sequence recited in SEQ ID NO:2 or 4, or a variant thereof, wherein the polypeptide has sphingosine-1-phosphate lyase activity.

Within a further aspect, the present invention provides isolated polynucleotides comprising at least 100 nucleotides complementary to a sequence recited in SEQ ID NO:1 or 3.

Within other aspects, methods are provided for preparing a sphingosine-1-phosphate lyase, comprising culturing a host cell transformed or transfected with a polynucleotide as described above under conditions promoting expression of the polynucleotide and recovering a sphingosine-1-phosphate lyase.

In further aspects, the present invention provides methods for identifying an agent that modulates sphingosine-1-phosphate lyase activity. In one such aspect, the method comprises: (a) contacting a candidate agent with cells that express sphingosine-1-phosphate lyase; and (b) subsequently measuring the level of sphingosine-1-phosphate lyase or mRNA encoding sphingosine-1-phosphate lyase in the cells, relative to a predetermined level in the absence of candidate agent. Within another such aspect, the method comprises: (a) contacting a candidate agent with a polypeptide comprising a sequence recited in any one of SEQ ID NOs: 2, 4, 6 or 8, or a variant of such a sequence having sphingosine-1-phosphate lyase activity, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and (b) subsequently measuring the ability of the polypeptide to degrade sphingosine-1-phosphate or a derivative thereof, relative to an ability in the absence of candidate agent. The step of contacting may be performed by incubating a cell expressing the polypeptide with the candidate modulator, and the step of measuring the ability to degrade sphingosine-1-phosphate may be performed using an *in vitro* assay and a cellular extract.

The present invention further provides pharmaceutical compositions comprising an agent that modulates sphingosine-1-phosphate lyase

activity in combination with a pharmaceutically acceptable carrier. Such agents preferably inhibit sphingosine-1-phosphate lyase activity. Such inhibition may be achieved by inhibiting expression of an endogenous SPL gene, or by inhibiting the ability of an endogenous SPL to degrade sphingosine-1-phosphate. Within certain preferred embodiments, a modulating agent comprises a polynucleotide or an antibody or an antigen-binding fragment thereof.

Within still further aspects, the present invention provides methods for modulating sphingosine-1-phosphate activity, comprising contacting a sphingosine-1-phosphate lyase with an effective amount of an agent that modulates sphingosine-1-phosphate lyase activity, wherein the step of contacting is performed under conditions and for a time sufficient to allow the agent and the sphingosine-1-phosphate lyase to interact. To modulate sphingosine-1-phosphate lyase activity in a cell, a cell expressing sphingosine-1-phosphate may be contacted with such an agent.

Within related aspects, the present invention provides methods for inhibiting the growth of a cancer cell, comprising contacting a cancer cell with an agent that inhibits sphingosine-1-phosphate lyase activity. In a preferred embodiment, the cancer cell is a breast cancer cell.

The present invention also provides methods for inhibiting the development and/or metastasis of a cancer in a mammal, comprising administering to a mammal an agent that inhibits sphingosine-1-phosphate lyase activity. Within certain embodiments, an agent may comprise, or be linked to, a targeting component, such as an anti-tumor antibody or a component that binds to an estrogen receptor.

Within other aspects, methods for diagnosing cancer in a mammal are provided, comprising detecting an alteration in an endogenous sphingosine-1-phosphate lyase gene in a sample obtained from a mammal, and therefrom diagnosing a cancer in the mammal. In certain embodiments the cancer is breast cancer and the sample is a breast tumor biopsy.

In related aspects, the present invention provides methods for evaluating a cancer prognosis, comprising determining the presence or absence of

an alteration in an endogenous sphingosine-1-phosphate lyase gene in a sample obtained from a mammal afflicted with cancer, and therefrom determining a prognosis.

The present invention further provides isolated antibodies that bind to a polypeptide having a sequence recited in any one of SEQ ID NOs: 2, 4 or 6. Such antibodies may be polyclonal or monoclonal, and may inhibit the ability of a polypeptide having a sequence recited in any one of SEQ ID NOs: 2, 4 or 6 to degrade sphingosine-1-phosphate.

In still further aspects, the present invention provides methods for detecting sphingosine-1-phosphate lyase in a sample, comprising: (a) contacting a sample with an antibody as described above under conditions and for a time sufficient to allow the antibody to bind to sphingosine-1-phosphate lyase; and (b) detecting in the sample the presence of sphingosine-1-phosphate lyase bound to the antibody.

Kits for use in the above methods are also provided. A kit for detecting sphingosine-1-phosphate lyase in a sample comprises an antibody as described above and a buffer or detection reagent. A kit for detecting an alteration in a sphingosine-1-phosphate gene in a sample comprises a polynucleotide and a detection reagent.

Within further aspects, the present invention provides transgenic animals in which sphingosine-1-phosphate lyase activity is reduced, and cell lines derived from such transgenic animals.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figures 1A-1C present the sequence of a *S. cerevisiae* polynucleotide encoding a representative SPL polypeptide

Figures 2A and 2B present the sequence of a *C. elegans* polynucleotide encoding a representative SPL polypeptide.

Figures 3A and 3B present the sequence of a *Mus musculus* polynucleotide encoding a representative SPL polypeptide.

Figure 4 presents a comparison of the endogenous SPL genomic sequences from *C. elegans*, yeast and mouse.

Figure 5 is a photograph showing the growth of yeast cells grown to saturation in liquid culture and then plated on YPD with (top plate) and without (lower plate) 50μM sphingosine. On each plate, the top row of cells is BST1Δ (JS16, which is a variation of SGP3 (*leu2-3,112 trpl ura3-52 his3 ade8 rasI::HIS3*) in which the *BST1* gene has been replaced by a G418-resistant marker, *NEO*). The second row is JS16 transformed with vector alone. The third row and the bottom two rows (mBST1) show JS60 cells (JS16[pYES-mouse*SPL*]) and the fourth row (ceBST1) shows JS61 cells (JS16[pYES2-*C. elegansBST1*]). The fifth row on each plate (BST1-WT) shows the growth of the wildtype SGP3 strain.

Figure 6A is an autoradiogram showing the products of an SPL assay performed on extracts obtained from JS16 transformed with JS29=pYES2-yeast *BST1* (ytBST1), JS60=pYES2-mouseSPL (mBST1) or pYES2 without insert (vehicle control). Figure 6B is a histogram depicting the activity in the strains shown in Figure 6A, as determined by scraping a TLC plate as shown in Figure 6A and assessing the level of radioactivity.

Figure 7 is an autoradiogram depicting the results of a Northern blot analysis of the level of mouse SPL in various mouse tissues, as indicated.

Figures 8A-8C present a sequence of a human polynucleotide encoding a representative SPL polypeptide.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and therapy of cancers such as breast cancer. The invention is more particularly related to sphingosine-1-phosphate

lyase (SPL) polypeptides, which have the ability to cleave sphingosine-1-phosphate into inactive metabolites, and to polynucleotides encoding such polypeptides. Sphingosine-1-phosphate is an endogenous tumor-suppressor lipid that potently inhibits breast cancer cell growth and invasiveness, while not affecting the growth of non-tumor cells (*see* Sadahira et al., *Proc. Natl. Acad. Sci. USA 89*:9686-90, 1992). *In vivo,* SPL catalyzes the cleavage of sphingosine-1-phosphate at the C_{2-3} carbon bond to yield a long chain aldehyde and ethanolamine phosphate, the final step in the degradation of all higher order sphingolipids. Agents that decrease the expression or activity of endogenous SPL polypeptides are encompassed by the present invention. Such modulating agents may be identified using methods described herein and used, for example, in cancer therapy. It has also been found, within the context of the present invention, that the detection of alterations in an endogenous SPL sequence can be used to diagnose cancer, and to assess the prognosis for recovery. The present invention further provides such diagnostic methods and kits.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length endogenous (*i.e.*, native) SPL proteins and variants of endogenous sequences. "Variants" are polypeptides that differ in sequence from a native SPL only in substitutions, deletions and/or other modifications, such that the variant retains SPL activity, which may be determined using a representative method described herein. Within an SPL polypeptide variant, amino acid substitutions are preferably made at no more than 50% of the amino acid residues in the native polypeptide, and more preferably at no more than 25% of the amino acid residues. Such substitutions are preferably conservative. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. Substitutions, deletions and/or amino acid

additions may be made at any location(s) in the polypeptide, provided that the modification does not diminish the SPL activity of the variant. Thus, a variant may comprise only a portion of a native SPL sequence. In addition, or alternatively, variants may contain additional amino acid sequences (such as, for example, linkers, tags and/or ligands), preferably at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification, detection or cellular uptake of the polypeptide.

The SPL activity of an SPL polypeptide may generally be assessed using an *in vitro* assay that detects the degradation of labeled substrate (*i.e.*, sphingosine-1-phosphate, or a derivative thereof). Within such assays, pyridoxal 5'-phosphate is a requirement for SPL activity. In addition, the reaction generally proceeds optimally at pH 7.4-7.6 and requires chelators due to sensitivity toward heavy metal ions. The substrate should be a D-*erythro* isomer, but in derivatives of sphingosine-1-phosphate the type and chain length of sphingoid base may vary. In general, an assay as described by Van Veldhoven and Mannaerts, *J. Biol. Chem. 266*:12502-07, 1991 may be employed. Briefly, a solution (*e.g.*, a cellular extract) containing the polypeptide may be incubated with 40 μM substrate at 37°C for 1 hour in the presence of, for example, 50 mM sucrose, 100 mM K-phosphate buffer pH 7.4, 25 mM NaF, 0.1% (w/v) Triton X-100, 0.5 mM EDTA, 2 mM DTT, 0.25 mM pyridoxal phosphate. Reactions may then be terminated and analyzed by thin-layer chromatography to detect the formation of labeled fatty aldehydes and further metabolites. In general, a polypeptide has SPL activity if, within such an assay: (1) the presence of 2 - 50 μg polypeptide (or 0.1 - 10 mg/mL) results in a statistically significant increase in the level of substrate degradation, preferably a two-fold increase, relative to the level observed in the absence of polypeptide; and (2) the increase in the level of substrate degradation is pyridoxal 5'-phosphate dependent.

Within certain embodiments, an *in vitro* assay for SPL activity may be performed using cellular extracts prepared from cells that express the polypeptide of interest. Preferably, in the absence of a gene encoding an SPL polypeptide, such cells do not produce a significant amount of endogenous SPL

(*i.e.*, a cellular extract should not contain a detectable increase in the level of SPL, as compared to buffer alone without extract). It has been found, within the context of the present invention, that yeast cells containing deletion of the SPL gene (*BST1*) are suitable for use in evaluating the SPL activity of a polypeptide. *bst1*Δ cells can be generated from *S. cerevisiae* using standard techniques, such as PCR, as described herein. A polypeptide to be tested for SPL activity may then be expressed in *bst1*Δ cells, and the level of SPL activity in an extract containing the polypeptide may be compared to that of an extract prepared from cells that do not express the polypeptide. For such a test, a polypeptide is preferably expressed on a high-copy yeast vector (such as pYES2, which is available from Invitrogen) yielding more than 20 copies of the gene per cell. In general, a polypeptide has SPL activity if, when expressed using such a vector in a *bst1*Δ cell, a cellular extract results in a two-fold increase in substrate degradation over the level observed for an extract prepared from cells not expressing the polypeptide.

A further test for SPL activity may be based upon functional complementation in the *bst1*Δ strain. It has been found, within the context of the present invention, that *bst1*Δ cells are highly sensitive to D-*erythro*-sphingosine. In particular, concentrations as low as 10 μM sphingosine completely inhibit the growth of *bst1*Δ cells. Such a level of sphingosine has no effect on the growth of wildtype cells. A polypeptide having SPL activity as provided above significantly diminishes (*i.e.*, by at least two fold) the sphingosine sensitivity when expressed on a high-copy yeast vector yielding more than 20 copies of the gene per cell.

In general, SPL polypeptides, and polynucleotides encoding such polypeptides, may be prepared using any of a variety of techniques that are well known in the art. For example, a DNA sequence encoding native SPL may be prepared by amplification from a suitable cDNA or genomic library using, for example, polymerase chain reaction (PCR) or hybridization techniques. Libraries may generally be prepared and screened using methods well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring

Harbor, NY, 1989. cDNA libraries may be prepared from any of a variety of sources known to contain enzymes having SPL activity. SPL activity is ubiquitous with regard to species and mammalian tissues, with the exception of platelets, in which SPL activity is notably absent. In rat tissues, the highest levels of activity have been demonstrated in intestinal mucosa, liver and Harderian gland, with low activity in skeletal muscle and heart. Activity has also been demonstrated in a number of human (hepatoma cell line HB 8065, cervical carcinoma HeLa), mouse (hepatoma line BW1, mouse embryo 3T3-L1, Swiss 3T3 cells) and other cell lines, as well as in human cultured fibroblasts. Preferred cDNA libraries may prepared from human liver, intestine or brain tissues or cells. Other libraries that may be employed will be apparent to those of ordinary skill in the art. Primers for use in amplification may be readily designed based on the sequence of a native SPL polypeptide or polynucleotide, as provided herein.

Alternatively, an endogenous SPL gene may be identified using a screen for cDNAs that complement the *BST1* deletion in yeast. A cDNA expression library may be generated using a regulatable yeast expression vector (*e.g.*, pYES, which is availablve from Invitrogen, Inc.) and standard techniques. A yeast *bst1*Δ strain may then be transformed with the cDNA library, and endogenous cDNAs having the ability to functionally complement the yeast lyase defect (*i.e.*, restore the ability to grow in the presence of D-*erythro*-sphingosine) may be isolated.

An endogenous SPL gene may also be identified based on cross-reactivity of the protein product with anti-SPL antibodies, which may be prepared as described herein. Such screens may generally be performed using standard techniques (*see* Huynh et al., "Construction and Screening cDNA Libraries in λgt11," *in* D.M. Glover, ed., *DNA Cloning: A Practical Approach*, 1:49-78, 1984 (IRL Press, Oxford)).

Polynucleotides encompassed by the present invention include DNA and RNA molecules that comprise an endogenous SPL gene sequence. Such polynucleotides include those that comprise a sequence recited in any one of SEQ ID NOs:1, 3, 5 and 7. Also encompassed are other polynucleotides that

encode an SPL amino acid sequence provided in any one of SEQ ID NOs: 2, 4, 6 and 8, as well as polynucleotides that encode variants of a native SPL sequence that retain SPL activity. Polynucleotides that are substantially homologous to a sequence complementary to an endogenous SPL gene are also within the scope of the present invention. "Substantial homology," as used herein refers to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide complementary to a sequence provided in SEQ ID NO:1 or SEQ ID NO:3, provided that the encoded SPL polypeptide variant retains SPL activity. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50-65°C, 5X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Nucleotide sequences that, because of code degeneracy, encode a polypeptide encoded by any of the above sequences are also encompassed by the present invention.

Polypeptides of the present invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells are bacteria, yeast, insect or mammalian cells, and more preferably the host cells are *S. cerevisiae bst1*Δ cells. The recombinant DNA may be cloned into any expression vector suitable for use within the host cell and transfected into the host cell using techniques well known to those of ordinary skill in the art. A suitable expression vector contains a promoter sequence that is active in the host cell. A tissue-specific or conditionally active promoter may also be used. Preferred promoters express the polypeptide at high levels.

Optionally, the construct may contain an enhancer, a transcription terminator, a poly(A) signal sequence, a bacterial or mammalian origin of replication and/or a selectable marker, all of which are well known in the art. Enhancer sequences may be included as part of the promoter region or separately. Transcription terminators are sequences that stop RNA polymerase-mediated transcription. The poly(A) signal may be contained within the termination sequence or incorporated separately. A selectable marker includes any gene that confers a phenotype on the host cell that allows transformed cells to be identified.

Such markers may confer a growth advantage under specified conditions. Suitable selectable markers for bacteria are well known and include resistance genes for ampicillin, kanamycin and tetracycline. Suitable selectable markers for mammalian cells include hygromycin, neomycin, genes that complement a deficiency in the host (*e.g.*, thymidine kinase and TK$^-$cells) and others well known in the art. For yeast cells, one suitable selectable marker is URA3, which confers the ability to grow on medium without uracil.

DNA sequences expressed in this manner may encode a native SPL polypeptide (*e.g.*, human), or may encode portions or other variants of native SPL polypeptide. DNA molecules encoding variants of a native SPL may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides.

To generate cells that express a polynucleotide encoding an SPL polypeptide, cells may be transfected using any of a variety of techniques known in the art. Such transfection may result in stable transformants or may be transient. One suitable transfection technique is electroporation, which may be performed on a variety of cell types, including mammalian cells, yeast cells and bacteria, using commercially available equipment. Optimal conditions for electroporation (including voltage, resistance and pulse length) are experimentally determined for the particular host cell type, and general guidelines for optimizing electroporation may be obtained from manufacturers. Other suitable methods for transfection will depend upon the type of cell used (*e.g.*, the lithium acetate method for yeast), and will be apparent to those of ordinary skill in the art. Following transfection, cells may be maintained in conditions that promote expression of the polynucleotide within the cell. Appropriate conditions depend upon the expression system and cell type, and will be apparent to those skilled in the art.

SPL polypeptides may be expressed in transfected cells by culturing the cell under conditions promoting expression of the transfected polynucleotide. Appropriate conditions will depend on the specific host cell and

expression vector employed, and will be readily apparent to those of ordinary skill in the art. For commercially available expression vectors, the polypeptide may generally be expressed according to the manufacturer's instructions. For certain purposes, expressed polypeptides of this invention may be isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and/or affinity chromatography.

The present invention further provides antibodies that bind to an SPL polypeptide. Antibodies may function as modulating agents (as discussed further below) to inhibit or block SPL activity *in vivo*. Alternatively, or in addition, antibodies may be used within screens for endogenous SPL polypeptides or modulating agents, for purification of SPL polypeptides, for assaying the level of SPL within a sample and/or for studies of SPL expression. Such antibodies may be polyclonal or monoclonal, and are generally specific for one or more SPL polypeptides and/or one or more variants thereof. Within certain preferred embodiments, antibodies are polyclonal.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising an SPL polypeptide or antigenic portion thereof is initially injected into a suitable animal (*e.g.*, mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule incorporating one or more booster immunizations. The use of rabbits is preferred. To increase immunogenicity, an immunogen may be linked to, for example, glutaraldehyde or keyhole limpet hemocyanin (KLH). Following injection, the animals are bled periodically to obtain post-immune serum containing polyclonal anti-SPL antibodies. Polyclonal antibodies may then be purified from such antisera by, for example, affinity chromatography using an SPL polypeptide or antigenic portion

thereof coupled to a suitable solid support. Such polyclonal antibodies may be used directly for screening purposes and for Western blots.

More specifically, an adult rabbit (*e.g.*, NZW) may be immunized with 10 µg purified (*e.g.*, using a nickel-column) SPL polypeptide emulsified in complete Freund's adjuvant (1:1 v/v) in a volume of 1mL. Immunization may be achieved via injection in at least six different subcutaneous sites. For subsequent immunizations, 5 µg of an SPL polypeptide may be emulsified in in complete Freund's adjuvant and injected in the same manner. Immunizations may continue until a suitable serum antibody titer is achieved (typically a total of about three immunizations). The rabbit may be bled immediately before immunization to obtain pre-immune serum, and then 7-10 days following each immunization.

For certain embodiments, monoclonal antibodies may be desired. Monoclonal antibodies may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may

then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

As noted above, the present invention provides agents that modulate, preferably inhibit, the expression (transcription or translation), stability and/or activity of an SPL polypeptide. To identify such a modulating agent, any of a variety of screens may be performed. Candidate modulating agents may be obtained using well known techniques from a variety of sources, such as plants, fungi or libraries of chemicals, small molecules or random peptides. Antibodies that bind to an SPL polypeptide, and anti-sense polynucleotides that hybridize to a polynucleotides that encodes an SPL, may be candidate modulating agents. Preferably, a modulating agent has a minimum of side effects and is non-toxic. For some applications, agents that can penetrate cells are preferred.

Screens for modulating agents that decrease SPL expression or stability may be readily performed using well known techniques that detect the level of SPL protein or mRNA. Suitable assays include RNAse protection assays, *in situ* hybridization, ELISAs, Northern blots and Western blots. Such assays may generally be performed using standard methods (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). For example, to detect mRNA encoding SPL, a nucleic acid probe complementary to all or a portion of the SPL gene sequence may be employed in a Northern blot analysis of mRNA prepared from suitable cells. To detect SPL protein, a reagent that binds to the protein (typically an antibody, as described herein) may be employed within an ELISA or Western assay. Following binding, a reporter group suitable for direct or indirect detection of the reagent is employed (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes (*e.g.*, horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to

directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

To use such assays for identifying a modulating agent, the level of SPL protein or mRNA may be evaluated in cells treated with one or more candidate modulating agents. An increase or decrease in SPL levels may be measured by evaluating the level of SPL mRNA and/or protein in the presence and absence of candidate modulating agent. For example, an antisense modulating agent may be evaluated by assaying the effect on SPL levels. Suitable cells for use in such assays include the breast cancer cell lines MCF-7 (ATCC Accession Number HTB-22) and MDA-MB-231 (ATCC Accession Number HTB-26). A candidate modulator may be tested by transfecting the cells with a polynucleotide encoding the candidate and evaluating the effect of expression of the polynucleotide on SPL levels. Alternatively, the cells may be contacted with a candidate modulator, typically in an amount ranging from about 10 nM to about 10 mM. A candidate that results in a statistically significant change in the level of SPL mRNA and/or protein is a modulating agent.

Alternatively, or in addition, a candidate modulating agent may be tested for the ability to inhibit SPL activity, using an *in vitro* assay as described herein (*see* Van Veldhoven and Mannaerts, *J. Biol. Chem.* *266*:12502-07, 1991) that detects the degradation of labeled substrate (*i.e.*, sphingosine-1-phosphate, or a derivative thereof). Briefly, a solution (*e.g.*, a cellular extract) containing an SPL polypeptide (*e.g.*, 10 nM to about 10 mM) may be incubated with a candidate modulating agent (typically 1 nM to 10 mM, preferably 10 nM to 1 mM) and a substrate (*e.g.*, 40 µM) at 37°C for 1 hour in the presence of, for example, 50 mM sucrose, 100 mM K-phosphate buffer pH 7.4, 25 mM NaF, 0.1% (w/v) Triton X-100, 0.5 mM EDTA, 2 mM DTT, 0.25 mM pyridoxal phosphate. Reactions may then be terminated and analyzed by thin-layer chromatography to detect the formation of labeled fatty aldehydes and further metabolites. A modulating agent (*e.g.*, an antibody) that inhibits SPL activity results in a statistically significant decrease in the degradation of sphingosine-1-phosphate, relative to the level of

degradation in the absence of modulating agent. Such modulating agents may be used to inhibit SPL activity in a cell culture or a mammal, as described below.

A modulating agent may additionally comprise, or may be associated with, a targeting component that serves to direct the agent to a desired tissue or cell type. As used herein, a "targeting component" may be any substance (such as a compound or cell) that, when linked to a compound enhances the transport of the compound to a target tissue, thereby increasing the local concentration of the compound. Targeting components include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting components include hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and other drugs and proteins that bind to a desired target site. In particular, anti-tumor antibodies and compounds that bind to an estrogen receptor may serve as targeting components. An antibody employed in the present invention may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')2, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage may be via any suitable covalent bond using standard techniques that are well known in the art. Such linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers.

For *in vivo* use, a modulating agent as described herein is generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more modulating agents in combination with a physiologically acceptable carrier. To prepare a pharmaceutical composition, an effective amount of one or more modulating agents is mixed with any pharmaceutical carrier(s) known to those skilled in the art to be suitable for the particular mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for

example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof. In addition, other pharmaceutically active ingredients (including other anti-cancer agents) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

A modulating agent may be prepared with carriers that protect it against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. An amount that, following administration, inhibits, prevents or delays the progression and/or metastasis of a cancer is considered effective. Preferably, the amount administered is sufficient to result in regression, as indicated by 50% mass or by scan dimensions. The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect

while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

As an alternative to direct administration of a modulating agent, a polynucleotide encoding a modulating agent may be administered. Such a polynucleotide may be present in a pharmaceutical composition within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, and colloidal dispersion systems such as liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal, as described above). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-49, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Another delivery system for polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preparation and use of liposomes is well known to those of ordinary skill in the art.

Within certain aspects of the present invention, one or more modulating agents may be used to modulate SPL expression and/or activity *in vitro*, in a cell or in a mammal. *In vitro*, an SPL polypeptide may be contacted with a modulating agent that inhibits SPL activity (*e.g.*, certain antibodies). For use within a cell or a mammal, such modulation may be achieved by contacting a target cell with an effective amount of a modulating agent, as described herein. Administration to a mammal may generally be achieved as described above.

As noted above, inhibition of SPL expression and/or activity provides a method for inhibiting the growth (*i.e.*, proliferation) of a cancer cell,

either in culture or in a mammal afflicted with cancer. *In vivo*, such inhibition may also be used to inhibit cancer development, progression and/or metastasis. Accordingly, one or more modulating agents as provided herein may be administered as described above to a mammal in need of anti-cancer therapy. Patients that may benefit from administration of a modulating agent are those afflicted with cancer. Such patients may be identified based on standard criteria that are well known in the art. Within preferred embodiments, a patient is afflicted with breast cancer, as identified based on tissue biopsy and microscopic evaluation, using techniques well known in the art. In particular, patients whose tumor cells contain a tissue-specific deletion and/or alteration within an endogenous SPL gene may benefit from administration of a modulating agent, as provided herein.

Within other aspects, the present invention provides methods and kits for diagnosing cancer and/or identifying individuals with a risk for metastasis that is higher or lower than average. It has been found, within the context of the present invention, that certain human tumor cells contain an altered SPL gene. In particular, certain brain tumor cells contain a deletion of residues 354 to 433 of the human SPL sequence indicated in Figure 8 and SEQ ID NO:4. Specific alterations present in other tumor cells, such as breast tumor cells, may be readily identified using standard techniques, such as PCR. Alterations that may be associated with a paticular tumor include amino acid deletions, insertions, substitutions and combinations thereof. Methods in which the presence or absence of such an alteration is determined may generally be used to detect cancer and to evaluate the prognosis for a patient known to be afflicted with cancer.

To detect an altered SPL gene, any of a variety of well-known techniques may be used including, but not limited to, PCR and hybridization techniques. Any sample that may contain cancerous cells may be assayed. In general, suitable samples are tumor biopsies. Within a preferred embodiment, a sample is a breast tumor biopsy.

Kits for diagnosing or evaluating the prognosis of a cancer generally comprise reagents for use in the particular assay to be employed. In

general, a kit of the present invention comprises one or more containers enclosing elements, such as probes, reagents or buffers, to be used in an assay. For example, a kit may contain one or more polynucleotide probes comprising at least 100 nucleotides, and preferably at least 200 nucleotides, complementary to an SPL mRNA. Such probe(s) may be used to detect an altered SPL gene by hybridization. For example, a kit may contain one probe that hybridizes to a region of an SPL gene that is not generally altered in tumors (a control) and a second probe that hybridizes to a region commonly deleted in breast cancer. A sample that contains mRNA that hybridizes to the first probe, and not to the second (using standard techniques) contains an altered SPL gene. Suitable control probes include probes that hybridize to a portion of the SPL gene outside of the commonly deleted region encoding amino acid resides 354 to 433; suitable probes for an altered region include probes that hybridize to a portion of the SPL gene that encodes amino acid residues 354 to 433. Alternatively, a kit may comprise one or more primers for PCR analyses, which may be readily designed based upon the sequences provided herein by those of ordinary skill in the art. Optionally, a kit may further comprise one or more solutions, compounds or detection reagents for use within an assay as described above.

In a related aspect of the present invention, kits for detecting SPL are provided. Such kits may be designed for detecting the level of SPL or nucleic acid encoding SPL within a sample, or may detect the level of SPL activity as described herein. A kit for detecting the level of SPL, or nucleic acid encoding SPL, typically contains a reagent that binds to the SPL protein, DNA or RNA. To detect nucleic acid encoding SPL, the reagent may be a nucleic acid probe or a PCR primer. To detect SPL protein, the reagent is typically an antibody. The kit may also contain a reporter group suitable for direct or indirect detection of the reagent as described above.

Within further aspects, the present invention provides transgenic mammals in which SPL activity is reduced, compared to a wild-type animal. Such animals may contain an alteration, insertion or deletion in an endogenous SPL gene, or may contain DNA encoding a modulating agent that inhibits

expression or activity of an SPL gene. Transgenic animals may be generated using techniques that are known to those of ordinary skill in the art. For example, a transgenic animal containing an insertion or deletion in the coding region for the SPL gene may be generated from embryonic stem cells, using standard techniques. Such stem cells may be generated by first identifying the full genomic sequence of the gene encoding the SPL, and then creating an insertion or deletion in the coding region in embryonic stem cells. Alternatively, appropriate genetically altered embryonic stem cells may be identified from a bank. Using the altered stem cells, hybrid animals may be generated with one normal SPL gene and one marked, abnormal gene. These hybrids may be mated, and homozygous progeny identified.

Transgenic aminals may be used for a variety of purposes, which will be apparent to those of ordinary skill in the art. For example, such animals may be used to prepare cell lines from different tissues, using well known techniques. Such cell lines may be used, for example, to evaluate the effect of the alteration, and to test various candidate modulators.

Summary of Sequence Listing

SEQ ID NO:1 is cDNA sequence encoding mouse endogenous SPL.

SEQ ID NO:2 is amino acid sequence of mouse endogenous SPL.

SEQ ID NO:3 is cDNA sequence encoding human endogenous SPL.

SEQ ID NO:4 is amino acid sequence of human endogenous SPL.

SEQ ID NO:5 is cDNA sequence encoding *C. elegans* endogenous SPL.

SEQ ID NO:6 is amino acid sequence of *C. elegans* endogenous SPL.

SEQ ID NO:7 is cDNA sequence encoding yeast endogenous SPL.

SEQ ID NO:8 is amino acid sequence of yeast endogenous SPL.

SEQ ID NO:9 is cDNA sequence encoding an altered human SPL.

SEQ ID NO:10 is amino acid sequence of an altered human SPL.

The following Examples are offered by way of illustration and not by way of limitation.

5

EXAMPLES

Example 1

Isolation and Characterization of SPL cDNA from Yeast

This Example illustrates the preparation of an *S. cerevisiae* cDNA molecule encoding an endogenous SPL polypeptide.

Wild-type yeast cells (SGP3 (Garrett and Broach, *Genes and Dev. 3*:1336-1348, 1989); *leu2-3,112 trp1 ura3-52 his3 ade8 ras1::HIS3*) were transformed with a yeast genomic library carried on the pRS202 high-copy shuttle vector (Sikorski and Heiter, *Genetics 122*:19-27, 1989) containing a selectable nutritional marker (*URA3*). pRS202 is a modified version of the pRS306 vector, into which a 2 micron plasmid piece was inserted. Inserts from this library are approximately 6-8 kb in length. Wild type yeast were transformed with the high copy library as described by Ito et al., *J. Bact. 153*:163-68, 1983, selected for uracil prototrophy (*i.e.*, the ability to grow on medium lacking uracil), and transformants were pooled and replated at a concentration of 10^6 cells per plate onto 1 mM D-*erythro*-sphingosine plates.

Six transformants which grew large colonies on 1mM D-*erythro*-sphingosine plates were grown in selective medium, and control SGP3 colonies were grown in minimal medium, at 30°C until saturated. Absorbance at 660 nm was used to correct for small variations in cell concentration between cultures. Serial dilutions were performed, and cells were template-inoculated onto 1 mM D-*erythro*-sphingosine plates and incubated at 30°C for 48 hours.

The most highly represented insert, 13-1, was subcloned and sequenced, and named *BST1* (bestower of sphingosine tolerance; GenBank accession number U51031; *Saccharomyces cerevisiae* genome database accession number YDR294C). The *BST1* nucleotide sequence encodes a previously unknown predicted protein of 65,523 kilodaltons and 589 amino acids in length. This sequence is 23% identical to *gadA* and *gadB*, two nearly identical *E. coli* genes encoding glutamate decarboxylase (GAD), a pyridoxal-5'-phosphate-dependent enzyme which catalyzes synthesis of the neurotransmitter γ-amino

butyric acid. *BST1* has been localized to *S. cerevisiae* chromosome 4. The sequence of *BST1* is provided in Figure 1 and SEQ ID NO:7.

To explore the function of *BST1*, a deletion strain was created through homologous recombination using a *NEO* selectable marker (Wach et al., *Yeast 10*:1793-1808, 1994). Genomic BST1 was replaced with kanMX (Wach et al., *Yeast 10*:1793-1808, 1994), which confers resistance to G418. Disruption was confirmed using PCR amplification of genomic DNA from G418 resistant clones, using primers to genomic sequence just 5' and 3' to the region replaced by the disruption. Deletion of *BST1* and all subsequent biological studies were performed in both SGP3 and in JK93d (Hietman et al., *Proc. Natl. Acad. Sci. USA 88*:1948-52, 1991); *ura3-52 leu2-3,112 his4 trp1 rme1*). Heterozygous diploids were sporulated, and spores segregated 2:2 for G418 resistance. Both G418 resistant and sensitive progeny were viable, indicating that *BST1* is not an essential gene.

Analysis of GAD activity in cytosolic extracts from wild type, *BST1* overexpression and *bst1Δ* strains indicated that *BST1* does not encode the *S. cervisiae* homologue of GAD. However, deletion of *BST1* was associated with severe sensitivity to D-*erythro*-sphingosine. Concentrations as low as 10 μM sphingosine completely inhibited growth of *bst1Δ* strains but had no effect on the viability of wild type cells. In comparison to the control strain, the *bst1Δ* strain also demonstrated greater sensitivity to 100 μM phytosphingosine, the long chain base endogenous to *S. cerevisia*. No difference between the growth of wild type and *BST1* overexpression strains on phytosphingosine, which is only minimally toxic to wild type cells at this concentration, was observed.

To determine whether differences in sphingosine uptake or metabolism were responsible for these sensitivity differences, *BST1* wild type, overexpression and *bst1Δ* strains were exposed to [C3-^{3}H]labeled sphingosine (American Radiolabeled Chemical, Inc., St. Louis, MO), washed in sterile water and subjected to Bligh-Dyer extractions (Bligh and Dyer, *Can. J. Buichem. Physiol. 37*:911-17, 1959). There were no major differences in sphingosine

recovery among the three strains. However, the aqueous phase from the *bst1Δ* strain contained a ten-fold increase in radioactivity over that of control and *BST1* overexpression strains. Thin layer chromatography (TLC) analysis of the lipid fractions in butanol:acetic acid:water (3:1:1) revealed a sphingosine band which appeared equivalent in each strain.

Radioactive sphingosine-1-phosphate (S-1-P) was also observed in the extracts from the *bst1Δ* strain, but not in the wild type or *BST1* overexpression strains. This compound accumulated rapidly, reaching a plateau by 60 minutes. Three separate TLC conditions were used to confirm the presence of S-1-P. These conditions, along with the resulting RF values, are shown below:

butanol:water:acetic acid (3:1:1)

.47

chloroform:methanol:water (60:35:8)

.22

chloroform:methanol:water:acetic acid (30:30:2:5)

.33

Hyperaccumulation of S-1-P and hypersensitivity to D-*erythro*-sphingosine suggeset a failure to metabolize S-1-P, indicating that BST1 is a yeast SPL. To confirm this identification, lyase activity in *BST1* wild type, overexpression and deletion strains were evaluated as described by Veldhoven and Mannaerts, *J. Biol. Chem. 266*:12502-07, 1991, using unlabeled D-*erythro*-dihydrosphingosine-1-phosphate (Biomol, Plymouth Meeting, PA) and D-*erythro*-dihydrosphingosine [4,5-^{3}H]1-phosphate (American Radiolabeled Chemicals, Inc., St. Louis, MO). Specific activity was 100 mCi/mmol. SPL activity was found to correlate with *BST1* expression, confirming *BST1* to be the yeast homologue of sphingosine-1-phosphate lyase.

These results indication that *BST1* is a yeast SPL, and that SPL catalyzes a rate-limiting step in sphingolipid catabolism. Regulation of SPL activity may therefore result in regulation of intracellular S-1-P levels.

Example 2

Isolation and Characterization of SPL cDNA from *C. elegans* and Mouse

This Example illustrates the identification of endogenous SPL cDNAs from *C. elegans* and *Mus musculus*.

Comparison of the yeast *BST1* sequence to sequences within the GenBank database identified a full length gene from *C. elegans* that was identified during the systematic sequencing of the *C. elegans* genome. This sequence was found to encode SPL, and is shown in Figure 2 and SEQ ID NOs:5 and 6. This and other DNA homology searches described hereinwere performed via the National Center for Biotechnology Information website using BLAST search program.

Using both *S. cerevisiae* and *C. elegans* SPL sequences to search the EST database, an expressed sequence tag from early embryonic cells of the mouse (day 8 embryo, strain C57BL/6J) was identified. The cDNA clone containing this putative mouse SPL was purchased from Genome Systems, Inc (St. Louis, MO). Completion of the full length cDNA sequence revealed an 1709 bp open reading frame (Figure 3 and SEQ ID NOs:1 and 2). This mouse sequence showed significant homology to *BST1* and to other pyridoxal phosphate-binding enzymes such as glutamate decarboxylase, with greatest conservation surrounding the predicted pyridoxal phosphate-binding lysine (Figure 4). Since the two genes encoding mouse glutamate decarboxylase have been identified previously, and the identified sequence was unique and had no known function, it was a likely candidate mouse SPL gene.

To confirm the SPL activity of the mouse gene, a two step process was undertaken. First, the sequence was cloned into the high-copy yeast expression vector, pYES2 (Invitrogen, Inc., Carlsbad, CA), in which the gene of

interest is placed under control of the yeast GAL promoter and is, therefore, transcriptionally activated by galactose and repressed by glucose. pYES2 also contains the *URA3* gene (which provides transformants the ability to grow in media without uracil) and an ampicillin resistance marker and origin of replication functional in *E. coli*.

The expression vector containing the full-length mouse SPL gene was then introduced into the yeast *bst1Δ* strain whichn as noted above, is extremely sensitive to D-*erythro*-sphingosine, as a result of metabolism of sphingosine to S-1-P. S-1-P cannot be further degraded in the absence of SPL activity and overaccumulates, causing growth inhibition. Transformation was performed using the lithium acetate method (Ito et al., *J. Bact. 153*:163-68, 1983). Transformants were grown on medium containing 20g/L galactose and selected for uracil prototrophy.

Transformants were then evaluated for sphingosine resistance. Strains of interest were grown to saturation in liquid culture for 2-3 days. They were then resuspended in minimal medium, placed in the first row of a 96-well plate and diluted serially from 1:2 to 1:4000 across the plate. The cultures were then template inoculated onto a control plate (YPD) and a plate containing minimal synthetic media supplemented with 50 μM D-*erythro*-sphingosine (Sigma Chemical Co., St. Louis, MO) and 0.0015% NP40 (Sigma Chemical Co.). At this concentration of NP40, no effects on cell viability were observed. Plates were incubated at 30°C for two days and assessed visually for differences in growth. Transformants containing the mouse SPL gene were resistant to sphingosine present in galactose-containing plates (Figure 5). A strain transformed with vector alone remained sensitive to sphingosine. Therefore, the mouse SPL gene was capable of reversing the sphingosine-sensitive phenotype of a yeast *bst1Δ* strain.

In order to determine whether the mouse SPL gene was able to restore biochemical SPL activity to the *bst1Δ* strain, the untransformed *bst1Δ* strain, and the *bst1Δ* strain transformed with pYES2 containing either *BST1* or the

putative mouse SPL gene were grown to exponential phase (A_{600}=1.0) in either minimal (JS16) or uracil medium containing galactose as a carbon source. Whole cell extracts were prepared from each strain as described above, adjusted for protein concentration, and evaluated for sphingosine phosphate lyase activity as described above, using ^{3}H-dihydrosphingosine-1-phosphate (American Radiolabeled Chemicals, Inc., St. Louis, MO). Qualitative analysis of product was performed by autoradiography. Quantitative measurement was performed by scraping TLC plates and determining radioactivity present using a standard scintillation counter.

The results of the sphingosine phosphate lyase assays are shown in Figures 6A and 6B. Expression of both the yeast and mouse sequences restored SPL activity to the *bst1Δ* strain, whereas vector alone had no effect, confirming the identity of the mouse sequence as SPL.

To determine whether the expression of the mouse SPL transcript coincided with previously reported tissue-specific SPL activity in the mouse, total RNA was obtained from a variety of mouse tissues and probed with the complete mouse SPL cDNA sequence. Northern analysis was performed as described by Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201, 1980, using a full length mouse SPL cDNA probe labeled by random labeling technique (Cobianchi and Wilson, *Meth. Enzymol. 152*:94-110, 1987). This analysis revealed a pattern of expression consistent with the known SPL activity in various mouse tissues, providing further confirmation that this sequence encodes mouse SPL (Figure 7).

Example 3

Isolation and Characterization of Human SPL cDNA

This Example illustrates the identification of an endogenous human cDNA.

An EST database was searched using the mouse SPL sequence described herein. Two distinct EST sequences having strong homology to the mouse sequence were identified from human sources. One of these sequences corresponded to the C-terminus, and the other corresponded to the N-terminus.

Primers were designed based on these sequences, and a DNA fragment was amplified by PCR from a human expression library made from human glioblastoma multiforme tissue RNA. The fragment was sequenced and was shown to contain a deletion, so the primers were used to amplify the gene from human fibroblast RNA. This gene has the sequence provided in SEQ ID NO:3, and the sequence of the gene containing the deletion is provided in SEQ ID NO:9.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) a sequence recited in SEQ ID NO:1;

(b) a sequence recited in SEQ ID NO:3;

(c) nucleotide sequences that hybridize to a polynucleotide complementary to either of the foregoing sequences under moderately stringent conditions, wherein the nucleotide sequences encode polypeptides having sphingosine-1-phosphate lyase activity; and

(d) nucleotide sequences that encode a polypeptide encoded by any of the foregoing sequences.

2. An isolated polynucleotide encoding a polypeptide recited in SEQ ID NO:2, or a variant of such a polypeptide that has sphingosine-1-phosphate lyase activity.

3. An isolated polynucleotide encoding a polypeptide comprising a sequence recited in SEQ ID NO:4, or a variant of such a polypeptide that has sphingosine-1-phosphate lyase activity.

4. A recombinant expression vector comprising a polynucleotide according to any one of claims 1-3.

5. A host cell transformed or transfected with an expression vector according to claim 4.

6. An isolated polynucleotide comprising at least 100 nucleotides complementary to a sequence recited in SEQ ID NO:1 or 3.

7. A method for preparing a sphingosine-1-phosphate lyase, the method comprising culturing a host cell transformed or transfected with a polynucleotide according to any one of claims 1-3 under conditions promoting expression of the polynucleotide and recovering a sphingosine-1-phosphate lyase.

8. A polypeptide comprising an amino acid sequence encoded by a polynucleotide according to claim 1.

9. A polypeptide comprising an amino acid sequence recited in SEQ ID NO:2 or 4, or a variant thereof, wherein the polypeptide has sphingosine-1-phosphate lyase activity.

10. A method for identifying an agent that modulates sphingosine-1-phosphate lyase activity, comprising:

(a) contacting a candidate agent with cells that express sphingosine-1-phosphate lyase; and

(b) subsequently measuring the level of sphingosine-1-phosphate lyase or mRNA encoding sphingosine-1-phosphate lyase in the cells, relative to a predetermined level in the absence of candidate agent, and therefrom identifying an agent that modulates sphingosine-1-phosphate lyase activity.

11. A method for identifying an agent that modulates sphingosine-1-phosphate lyase activity, comprising:

(a) contacting a candidate agent with a polypeptide comprising a sequence recited in any one of SEQ ID NOs: 2, 4, 6 or 8, or a variant of such a sequence having sphingosine-1-phosphate lyase activity, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and

(b) subsequently measuring the ability of the polypeptide to degrade sphingosine-1-phosphate or a derivative thereof, relative to an ability in the absence

of candidate agent, and therefrom identifying an agent that modulates sphingosine-1-phosphate lyase activity.

12. A method according to claim 11, wherein the step of contacting is performed by incubating a cell expressing the polypeptide with the candidate modulator, and wherein the step of measuring the ability to degrade sphingosine-1-phosphate is performed using an *in vitro* assay and a cellular extract.

13. A pharmaceutical composition comprising an agent that modulates sphingosine-1-phosphate lyase activity in combination with a pharmaceutically acceptable carrier.

14. A composition according to claim 13, wherein the sphingosine-1-phosphate lyase comprises a sequence recited in any one of SEQ ID NOs:2, 4 or 6.

15. A composition according to claim 13, wherein the agent inhibits expression of an endogenous sphingosine-1-phosphate lyase gene.

16. A composition according to claim 15, wherein the agent comprises a polynucleotide.

17. A composition according to claim 13, wherein the agent inhibits the ability of an endogenous sphingosine-1-phosphate lyase to degrade sphingosine-1-phosphate.

18. A composition according to claim 17, wherein the agent comprises an antibody or an antigen-binding fragment thereof.

19. A method for modulating sphingosine-1-phosphate lyase activity, comprising contacting a sphingosine-1-phosphate lyase with an effective amount of an agent that modulates sphingosine-1-phosphate lyase activity, wherein the step of contacting is

performed under conditions and for a time sufficient to allow the agent and the sphingosine-1-phosphate lyase to interact.

20. A method according to claim 19, wherein the step of contacting is performed by incubating a cell expressing the polypeptide with the agent.

21. A method according to claim 19, wherein the agent inhibits expression of an endogenous sphingosine-1-phosphate lyase gene.

22. A method according to claim 21, wherein the agent comprises a polynucleotide.

23. A method according to claim 19, wherein the agent is capable of inhibiting the ability of a polypeptide comprising a sequence recited in any one of SEQ ID NOs: 2, 4 or 6 to degrade sphingosine-1-phosphate.

24. A method for inhibiting the growth of a cancer cell, comprising contacting a cancer cell with an agent that inhibits sphingosine-1-phosphate lyase activity.

25. A method according to claim 24, wherein the agent inhibits expression of an endogenous sphingosine-1-phosphate lyase gene.

26. A method according to claim 25, wherein the agent comprises a polynucleotide according to claim 6.

27. A method according to claim 24, wherein the agent is capable of inhibiting the ability of a polypeptide comprising a sequence recited in any one of SEQ ID NOs: 2, 4 or 6 to degrade sphingosine-1-phosphate.

28. A method according to claim 24, wherein the cancer cell is a breast cancer cell.

29. A method for inhibiting the development and/or metastasis of a cancer in a mammal, comprising administering to a mammal an agent that inhibits sphingosine-1-phosphate lyase activity.

30. A method according to claim 29, wherein the agent inhibits expression of an endogenous sphingosine-1-phosphate lyase gene.

31. A method according to claim 30, wherein the agent comprises a polynucleotide according to claim 6.

32. A method according to claim 29, wherein the agent is capable of inhibiting the ability of a polypeptide comprising a sequence recited in any one of SEQ ID NOs: 2, 4 or 6 to degrade sphingosine-1-phosphate.

33. A method according to claim 29, wherein the agent is linked to a targeting component.

34. A method according to claim 33, wherein the targeting component is an anti-tumor antibody.

35. A method according to claim 33, wherein the targeting component binds to an estrogen receptor.

36. A method according to claim 29, wherein the mammal is afflicted with breast cancer.

37. A method for diagnosing a cancer in a mammal, comprising detecting an alteration in an endogenous sphingosine-1-phosphate lyase gene in a sample obtained from a mammal, and therefrom diagnosing a cancer in the mammal.

38. A method according to claim 37, wherein the alteration is a deletion.

39. A method according to claim 37, wherein the cancer is breast cancer and the sample is a breast tumor biopsy.

40. A method for evaluating a cancer prognosis, comprising determining the presence or absence of an alteration in an endogenous sphingosine-1-phosphate lyase gene in a sample obtained from a mammal afflicted with cancer, and therefrom determining a prognosis.

41. A method according to claim 40, wherein the alteration is a deletion.

42. A method according to claim 41, wherein the deletion comprises amino acid residues 354-433 of SEQ ID NO:4.

43. A method according to claim 40, wherein the cancer is breast cancer and the sample is a breast tumor biopsy.

44. An isolated antibody that binds to a polypeptide having a sequence recited in any one of SEQ ID NOs: 2, 4 or 6.

45. A monoclonal antibody that binds to a polypeptide having a sequence recited in any one of SEQ ID NOs: 2, 4 or 6.

46. An antibody according to claim 44 or claim 45, wherein the antibody inhibits the ability of a polypeptide having a sequence recited in any one of SEQ ID NOs: 2, 4 or 6 to degrade sphingosine-1-phosphate.

47. A method for detecting sphingosine-1-phosphate lyase in a sample, comprising:

(a) contacting a sample with an antibody according to claim 44 or claim 45 under conditions and for a time sufficient to allow the antibody to bind to sphingosine-1-phosphate lyase; and

(b) detecting in the sample the presence of sphingosine-1-phosphate lyase bound to the antibody.

48. A kit for detecting sphingosine-1-phosphate lyase in a sample, comprising an antibody according to claim 44 or claim 45 and a buffer or detection reagent.

49. A kit for detecting an alteration in a sphingosine-1-phosphate gene in a sample, comprising a polynucleotide according to claim 6 and a detection reagent.

50. A transgenic animal in which sphingosine-1-phosphate lyase activity is reduced compared to a wild-type animal.

51. A cell line derived from a transgenic animal according to claim 50.

Fig. 1A

```
ATG AGT GGA GTA TCA AAT AAA ACA GTA TCA ATT AAT GGT TGG TAT GGC      48
Met Ser Gly Val Ser Asn Lys Thr Val Ser Ile Asn Gly Trp Tyr Gly
 1               5                  10                  15

ATG CCA ATT CAT TTA CTA AGG GAA GAA GGC GAC TTT GCC CAG TTT ATG      96
Met Pro Ile His Leu Leu Arg Glu Glu Gly Asp Phe Ala Gln Phe Met
            20                  25                  30

ATT CTA ACC ATC AAC GAA TTA AAA ATA GCC ATA CAT GGT TAC CTC AGA     144
Ile Leu Thr Ile Asn Glu Leu Lys Ile Ala Ile His Gly Tyr Leu Arg
        35                  40                  45

AAT ACC CCA TGG TAC AAC ATG TTG AAG GAT TAT TTG TTT GTG ATC TTT     192
Asn Thr Pro Trp Tyr Asn Met Leu Lys Asp Tyr Leu Phe Val Ile Phe
    50                  55                  60

TGT TAC AAG CTA ATA AGT AAT TTT TTT TAT CTG TTG AAA GTT TAT GGG     240
Cys Tyr Lys Leu Ile Ser Asn Phe Phe Tyr Leu Leu Lys Val Tyr Gly
65                  70                  75                  80

CCG GTG AGG TTA GCA GTG AGA ACA TAC GAG CAT AGT TCC AGA AGA TTG     288
Pro Val Arg Leu Ala Val Arg Thr Tyr Glu His Ser Ser Arg Arg Leu
                85                  90                  95

TTT CGT TGG TTA TTG GAC TCA CCA TTT TTG AGG GGT ACC GTA GAA AAG     336
Phe Arg Trp Leu Leu Asp Ser Pro Phe Leu Arg Gly Thr Val Glu Lys
            100                 105                 110

GAA GTC ACA AAG GTC AAA CAA TCG ATC GAA GAC GAA CTA ATT AGA TCG     384
Glu Val Thr Lys Val Lys Gln Ser Ile Glu Asp Glu Leu Ile Arg Ser
        115                 120                 125

GAC TCT CAG TTA ATG AAT TTC CCA CAG TTG CCA TCC AAT GGG ATA CCT     432
Asp Ser Gln Leu Met Asn Phe Pro Gln Leu Pro Ser Asn Gly Ile Pro
    130                 135                 140

CAG GAT GAT GTT ATT GAA GAG CTA AAT AAA TTG AAC GAC TTG ATA CCA     480
Gln Asp Asp Val Ile Glu Glu Leu Asn Lys Leu Asn Asp Leu Ile Pro
145                 150                 155                 160

CAT ACC CAA TGG AAG GAA GGA AAG GTC TCT GGT GCC GTT TAC CAC GGT     528
His Thr Gln Trp Lys Glu Gly Lys Val Ser Gly Ala Val Tyr His Gly
                165                 170                 175
```

Fig. 1B

```
GGT GAT GAT TTG ATC CAC TTA CAA ACA ATC GCA TAC GAA AAA TAT TGC      576
Gly Asp Asp Leu Ile His Leu Gln Thr Ile Ala Tyr Glu Lys Tyr Cys
            180                 185                 190

GTT GCC AAT CAA TTA CAT CCC GAT GTC TTT CCT GCC GTA CGT AAA ATG      624
Val Ala Asn Gln Leu His Pro Asp Val Phe Pro Ala Val Arg Lys Met
        195                 200                 205

GAA TCC GAA GTG GTT TCT ATG GTT TTA AGA ATG TTT AAT GCC CCT TCT      672
Glu Ser Glu Val Val Ser Met Val Leu Arg Met Phe Asn Ala Pro Ser
    210                 215                 220

GAT ACA GGT TGT GGT ACC ACA ACT TCA GGT GGT ACA GAA TCC TTG CTT      720
Asp Thr Gly Cys Gly Thr Thr Thr Ser Gly Gly Thr Glu Ser Leu Leu
225                 230                 235                 240

TTA GCA TGT CTG AGC GCT AAA ATG TAT GCC CTT CAT CAT CGT GGA ATC      768
Leu Ala Cys Leu Ser Ala Lys Met Tyr Ala Leu His His Arg Gly Ile
                245                 250                 255

ACC GAA CCA GAA ATA ATT GCT CCC GTA ACT GCA CAT GCT GGG TTT GAC      816
Thr Glu Pro Glu Ile Ile Ala Pro Val Thr Ala His Ala Gly Phe Asp
            260                 265                 270

AAA GCT GCT TAT TAC TTT GGC ATG AAG CTA CGC CAC GTG GAG CTA GAT      864
Lys Ala Ala Tyr Tyr Phe Gly Met Lys Leu Arg His Val Glu Leu Asp
        275                 280                 285

CCA ACG ACA TAT CAA GTG GAC CTG GGA AAA GTG AAA AAA TTC ATC AAT      912
Pro Thr Thr Tyr Gln Val Asp Leu Gly Lys Val Lys Lys Phe Ile Asn
    290                 295                 300

AAG AAC ACA ATT TTA CTG GTC GGT TCC GCT CCA AAC TTT CCT CAT GGT      960
Lys Asn Thr Ile Leu Leu Val Gly Ser Ala Pro Asn Phe Pro His Gly
305                 310                 315                 320

ATT GCC GAT GAT ATT GAA GGA TTG GGT AAA ATA GCA CAA AAA TAT AAA     1008
Ile Ala Asp Asp Ile Glu Gly Leu Gly Lys Ile Ala Gln Lys Tyr Lys
                325                 330                 335

CTT CCT TTA CAC GTC GAC AGT TGT CTA GGT TCC TTT ATT GTT TCA TTT     1056
Leu Pro Leu His Val Asp Ser Cys Leu Gly Ser Phe Ile Val Ser Phe
            340                 345                 350
```

Fig. 1C

```
ATG GAA AAG GCT GGT TAC AAA AAT CTG CCA TTA CTT GAC TTT AGA GTC   1104
Met Glu Lys Ala Gly Tyr Lys Asn Leu Pro Leu Leu Asp Phe Arg Val
      355                 360                 365

CCG GGA GTC ACC TCA ATA TCA TGT GAC ACT CAT AAA TAT GGA TTT GCA   1152
Pro Gly Val Thr Ser Ile Ser Cys Asp Thr His Lys Tyr Gly Phe Ala
   370                 375                 380

CCA AAA GGC TCG TCA GTT ATA ATG TAT AGA AAC AGC GAC TTA CGA ATG   1200
Pro Lys Gly Ser Ser Val Ile Met Tyr Arg Asn Ser Asp Leu Arg Met
385                 390                 395                 400

CAT CAG TAT TAC GTA AAT CCT GCT TGG ACT GGC GGG TTA TAT GGC TCT   1248
His Gln Tyr Tyr Val Asn Pro Ala Trp Thr Gly Gly Leu Tyr Gly Ser
                405                 410                 415

CCT ACA TTA GCA GGG TCC AGG CCT GGT GCT ATT GTC GTA GGT TGT TGG   1296
Pro Thr Leu Ala Gly Ser Arg Pro Gly Ala Ile Val Val Gly Cys Trp
            420                 425                 430

GCC ACT ATG GTC AAC ATG GGT GAA AAT GGG TAC ATT GAG TCG TGC CAA   1344
Ala Thr Met Val Asn Met Gly Glu Asn Gly Tyr Ile Glu Ser Cys Gln
      435                 440                 445

GAA ATA GTC GGT GCA GCA ATG AAG TTT AAA AAA TAC ATC CAG GAA AAC   1392
Glu Ile Val Gly Ala Ala Met Lys Phe Lys Lys Tyr Ile Gln Glu Asn
   450                 455                 460

ATT CCA GAC CTG AAT ATA ATG GGC AAC CCT AGA TAT TCA GTC ATT TCA   1440
Ile Pro Asp Leu Asn Ile Met Gly Asn Pro Arg Tyr Ser Val Ile Ser
465                 470                 475                 480

TTT TCT TCA AAG ACC TTG AAC ATA CAC GAA CTA TCT GAC AGG TTG TCC   1488
Phe Ser Ser Lys Thr Leu Asn Ile His Glu Leu Ser Asp Arg Leu Ser
                485                 490                 495

AAG AAA GGC TGG CAT TTC AAT GCC CTA CAA AAG CCG GTT GCA CTA CAC   1536
Lys Lys Gly Trp His Phe Asn Ala Leu Gln Lys Pro Val Ala Leu His
            500                 505                 510

ATG GCC TTC ACG AGA TTG AGC GCT CAT GTT GTG GAT GAG ATC TGC GAC   1584
Met Ala Phe Thr Arg Leu Ser Ala His Val Val Asp Glu Ile Cys Asp
      515                 520                 525
```

Fig. 1D

```
ATT TTA CGT ACT ACC GTG CAA GAG TTG AAG AGC GAA TCA AAT TCT AAA    1632
Ile Leu Arg Thr Thr Val Gln Glu Leu Lys Ser Glu Ser Asn Ser Lys
    530                 535                 540

CCA TCC CCA GAC GGA ACT AGC GCT CTA TAT GGT GTC GCC GGG AGC GTT    1680
Pro Ser Pro Asp Gly Thr Ser Ala Leu Tyr Gly Val Ala Gly Ser Val
545                 550                 555                 560

AAA ACT GCT GGC GTT GCA GAC AAA TTG ATT GTG GGA TTC CTA GAC GCA    1728
Lys Thr Ala Gly Val Ala Asp Lys Leu Ile Val Gly Phe Leu Asp Ala
                565                 570                 575

TTA TAC AAG TTG GGT CCA GGA GAG GAT ACC GCC ACC AAG TAG            1770
Leu Tyr Lys Leu Gly Pro Gly Glu Asp Thr Ala Thr Lys
            580                 585
```

Fig. 2A

C. elegans S-1-P Lyase Gene [1 to 1629] -> 1-phase Translation

DNA sequence 1629 b.p. ATGGATTTTGCA ... TTAACAGAGTGA linear

```
1/1                                 31/11
ATG GAT TTT GCA CTG GAG CAA TAT CAT AGT GCA AAG GAT TTG TTA ATA TTT GAG CTT CGA
M  D  F  A  L  E  Q  Y  H  S  A  K  D  L  L  I  F  E  L  R
61/21                               91/31
AAG TTC AAT CCA ATT GTT CTG GTT TCT AGT ACT ATT GTT GCA ACA TAC GTA CTC ACC AAT
K  F  N  P  I  V  L  V  S  S  T  I  V  A  T  Y  V  L  T  N
121/41                              151/51
CTG AGA CAT ATG CAT TTA GAT GAA ATG GGC ATC CGG AAA CGT TTG AGC ACT TGG TTT TTC
L  R  H  M  H  L  D  E  M  G  I  R  K  R  L  S  T  W  F  F
181/61                              211/71
ACC ACT GTA AAG CGT GTG CCT TTC ATC AGG AAA ATG ATT GAC AAA CAA CTA AAC GAA GTA
T  T  V  K  R  V  P  F  I  R  K  M  I  D  K  Q  L  N  E  V
241/81                              271/91
AAG GAC GAG CTT GAG AAA AGT CTG AGA ATT GTG GAT CGA AGC ACC GAA TAC TTC ACT ACA
K  D  E  L  E  K  S  L  R  I  V  D  R  S  T  E  Y  F  T  T
301/101                             331/111
ATC CCA AGC CAT TCA GTT GGA AGA ACT GAA GTA CTT CGC CTT GCT GCC ATC TAT GAT GAT
I  P  S  H  S  V  G  R  T  E  V  L  R  L  A  A  I  Y  D  D
361/121                             391/131
TTG GAA GGA CCA GCT TTT TTG GAA GGA AGA GTA TCT GGA GCA GTC TTC AAT AGA GAA GAC
L  E  G  P  A  F  L  E  G  R  V  S  G  A  V  F  N  R  E  D
421/141                             451/151
GAC AAG GAC GAA CGG GAG ATG TAT GAG GAG GTG TTC GGA AAA TTT GCC TGG ACC AAC CCA
D  K  D  E  R  E  M  Y  E  E  V  F  G  K  F  A  W  T  N  P
481/161                             511/171
CTT TGG CCA AAA TTG TTC CCT GGA GTG AGA ATC ATG GAG GCT GAA GTT GTT CGC ATG TGT
L  W  P  K  L  F  P  G  V  R  I  M  E  A  E  V  V  R  M  C
541/181                             571/191
TGT AAT ATG ATG AAT GGA GAT TCG GAG ACA TGT GGA ACT ATG TCA ACT GGT GGA TCC ATT
C  N  M  M  N  G  D  S  E  T  C  G  T  M  S  T  G  G  S  I
601/201                             631/211
TCA ATT CTT TTG GCG TGC CTG GCT CAT CGT AAT CGT CTT TTG AAA AGA GGA GAA AAG TAC
S  I  L  L  A  C  L  A  H  R  N  R  L  L  K  R  G  E  K  Y
661/221                             691/231
ACA GAG ATG ATT GTC CCA TCA TCC GTC CAT GCA GCG TTC TTC AAA GCT GCC GAA TGT TTC
T  E  M  I  V  P  S  S  V  H  A  A  F  F  K  A  A  E  C  F
```

Fig. 2B

C. elegans S-1-P Lyase Gene [1 to 1629] -> 1-phase Translation

```
721/241                                 751/251
CGT ATC AAA GTT CGC AAG ATT CCA GTT GAT CCT GTT ACT TTC AAA GTA GAC CTT GTC AAA
R   I   K   V   R   K   I   P   V   D   P   V   T   F   K   V   D   L   V   K
781/261                                 811/271
ATG AAA GCC GCA ATT AAC AAG AGA ACA TGT ATG TTA GTT GGA TCT GCT CCA AAC TTT CCA
M   K   A   A   I   N   K   R   T   C   M   L   V   G   S   A   P   N   F   P
841/281                                 871/291
TTT GGA ACT GTT GAT GAC ATT GAA GCT ATT GGA CAG CTA GGA CTT GAA TAT GAC ATC CCA
F   G   T   V   D   D   I   E   A   I   G   Q   L   G   L   E   Y   D   I   P
901/301                                 931/311
GTT CAT GTT GAT GCT TGT CTT GGT GGT TTC CTT CTT CCA TTC CTT GAA GAA GAC GAG ATT
V   H   V   D   A   C   L   G   G   F   L   L   P   F   L   E   E   D   E   I
961/321                                 991/331
CGC TAT GAC TTC CGT GTT CCT GGT GTA TCT TCG ATT TCT GCA GAT AGT CAC AAA TAC GGA
R   Y   D   F   R   V   P   G   V   S   S   I   S   A   D   S   H   K   Y   G
1021/341                                1051/351
CTC GCT CCA AAG GGG TCA TCA GTT GTT CTT TAT CGC AAT AAG GAA CTT CTT CAT AAT CAG
L   A   P   K   G   S   S   V   V   L   Y   R   N   K   E   L   L   H   N   Q
1081/361                                1111/371
TAC TTC TGT GAT GCT GAT TGG CAA GGA GGT ATC TAT GCA TCG GCT ACT ATG GAA GGA TCA
Y   F   C   D   A   D   W   Q   G   G   I   Y   A   S   A   T   M   E   G   S
1141/381                                1171/391
CGC GCT GGG CAC AAC ATT GCA CTT TGC TGG GCC GCA ATG CTT TAT CAC GCT CAG GAA GGA
R   A   G   H   N   I   A   L   C   W   A   A   M   L   Y   H   A   Q   E   G
1201/401                                1231/411
TAC AAG GCC AAT GCT AGA AAG ATT GTT GAC ACT ACA AGA AAG ATT AGA AAT GGA CTT TCA
Y   K   A   N   A   R   K   I   V   D   T   T   R   K   I   R   N   G   L   S
1261/421                                1291/431
AAC ATT AAG GGA ATC AAA TTA CAA GGG CCA AGT GAT GTT TGT ATT GTT AGC TGG ACA ACC
N   I   K   G   I   K   L   Q   G   P   S   D   V   C   I   V   S   W   T   T
1321/441                                1351/451
AAT GAT GGA GTT GAA CTC TAC AGA TTC CAT AAC TTC ATG AAG GAA AAA CAT TGG CAA CTG
N   D   G   V   E   L   Y   R   F   H   N   F   M   K   E   K   H   W   Q   L
1381/461                                1411/471
AAT GGA CTT CAA TTC CCA GCT GGA GTT CAT ATC ATG GTC ACT ATG AAT CAT ACT CAT CCT
N   G   L   Q   F   P   A   G   V   H   I   M   V   T   M   N   H   T   H   P
1441/481                                1471/491
GGA CTC GCT GAA GCT TTC GTC GCC GAT TGC AGA GCT GCA GTT GAG TTT GTC AAA AGC CAC
G   L   A   E   A   F   V   A   D   C   R   A   A   V   E   F   V   K   S   H
```

Fig. 2C

C. elegans S-1-P Lyase Gene [1 to 1629] -> 1-phase Translation

```
1501/501                                1531/511
AAA CCA TCG GAA TCC GAC AAG ACA AGT GAA GCA GCC ATC TAC GGA CTT GCT CAA AGT ATT.
K   P   S   E   S   D   K   T   S   E   A   A   I   Y   G   L   A   Q   S   I
1561/521                                1591/531
CCA GAC CGA TCG CTT GTT CAC GAG TTT GCT CAC AGC TAT ATC GAT GCT GTT TAT GCT TTA
P   D   R   S   L   V   H   E   F   A   H   S   Y   I   D   A   V   Y   A   L
1621/541
ACA GAG TGA
T   E   *
```

Fig. 3A

Mouse S-1-P Lyase Gene -> 1-phase Translation

DNA sequence 1707 b.p. ATGCCCGGAACC ... AAGCCCCGCTGA linear

```
1/1                                                31/11
ATG CCC GGA ACC GAC CTC CTC AAG CTG AAG GAC TTC GAG CCT TAT TTG GAG ATT TTG GAA
M  P  G  T  D  L  L  K  L  K  D  F  E  P  Y  L  I  L  E  S
61/21                                              91/31
TCT TAT TCC ACA AAA GCC AAG AAT TAT GTG AAT GGA TAT TGC ACC AAA TAT GAG CCC TGG
E  Y  S  T  K  A  K  N  Y  V  N  G  Y  C  T  K  Y  E  P  W
121/41                                             151/51
CAG CTC ATT GCG TGG AGT GTC CTG TGT ACT CTG CTG ATA GTC TGG GTG TAT GAG CTT ATC
Q  L  I  A  W  S  V  L  C  T  L  L  I  V  W  V  Y  E  L  I
181/61                                             211/71
TTC CAG CCA GAG AGT TTA TGG TCT CGG TTT AAA AAA AAA TTA TTT AAG CTT ATC AGG AAG
F  Q  P  E  S  L  W  S  R  F  K  K  K  L  F  K  L  I  R  K
241/81                                             271/91
ATG CCA TTT ATT GGA CGT AAG ATC GAA CAA CAG GTG AGC AAA GCC AAG AAG GAT CTT GTC
M  P  F  I  G  R  K  I  E  Q  Q  V  S  K  A  K  K  D  L  V
301/101                                            331/111
AAG AAC ATG CCA TTC CTA AAG GTG GAC AAG GAT TAT GTG AAA ACT CTG CCT GCT CAG GGT
K  N  M  P  F  L  K  V  D  K  D  Y  V  K  T  L  P  A  Q  G
361/121                                            391/131
ATG GGC ACA GCT GAG GTT CTG GAG AGA CTC AAG GAG TAC AGC TCC ATG GAT GGT TCC TGG
M  G  T  A  E  V  L  E  R  L  K  E  Y  S  S  M  D  G  S  W
421/141                                            451/151
CAA GAA GGG AAA GCC TCA GGA GCT GTG TAC AAT GGG GAA CCG AAG CTC ACG GAG CTG CTG
Q  E  G  K  A  S  G  A  V  Y  N  G  E  P  K  L  T  E  L  L
481/161                                            511/171
GTG CAG GCT TAT GGA GAA TTC ACG TGG AGC AAT CCA CTG CAT CCA GAT ATC TTC CCT GGA
V  Q  A  Y  G  E  F  T  W  S  N  P  L  H  P  D  I  F  P  G
541/181                                            571/191
TTG CGG AAG TTA GAG GCA GAA ATC GTT AGG ATG ACT TGT TCC CTC TTC AAT GGG GGA CCA
L  R  K  L  E  A  E  I  V  R  M  T  C  S  L  F  N  G  G  P
601/201                                            631/211
GAT TCC TGT GGA TGT GTG ACT TCT GGG GGA ACG GAA AGC ATC CTG ATG GCC TGC AAA GCT
D  S  C  G  C  V  T  S  G  G  T  E  S  I  L  M  A  C  K  A
661/221                                            691/231
TAC CGG GAC TTG GCG TTA GAG AAG GGG ATC AAA ACT CCA GAA ATT GTG GCT CCC GAG AGT
Y  R  D  L  A  L  E  K  G  I  K  T  P  E  I  V  A  P  E  S
721/241                                            751/251
GCC CAT GCT GCA TTC GAC AAA GCA GCT CAT TAT TTT GGG ATG AAG ATT GTC CGA GTT GCA
A  H  A  A  F  D  K  A  A  H  Y  F  G  M  K  I  V  R  V  A
```

Fig. 3B

Mouse S-1-P Lyase Gene -> 1-phase Translation

781/261 811/271
CTG AAA AAG AAC ATG GAG GTG GAT GTG CAG GCA ATG AAG AGA GCC ATC TCC AGG AAC ACA
L K K N M E V D V Q A M K R A I S R N T
841/281 871/291
GCT ATG CTG GTC TGT TCT ACC CCA CAG TTT CCT CAT GGT GTG ATG GAT CCT GTC CCC GAA
A M L V C S T P Q F P H G V M D P V P E
901/301 931/311
GTG GCC AAG TTA ACT GTC AGA TAT AAA ATC CCA CTC CAT GTG GAT GCT TGT CTG GGG GGC
V A K L T V R Y K I P L H V D A C L G G
961/321 991/331
TTC CTC ATT GTC TTC ATG GAG AAA GCA GGG TAC CCA CTG GAG AAA CCA TTT GAT TTC CGG
F L I V F M E K A G Y P L E K P F D F R
1021/341 1051/351
GTG AAA GGT GTG ACC AGC ATT TCA GCA GAT ACT CAT AAG TAT GGC TAT GCT CCT AAA GGT
V K G V T S I S A D T H K Y G Y A P K G
1081/361 1111/371
TCA TCA GTG GTG ATG TAC TCT AAC GAG AAG TAC AGG ACG TAC CAG TTC TTT GTT GGT GCA
S S V V M Y S N E K Y R T Y Q F F V G A
1141/381 1171/391
GAC TGG CAA GGT GGT GTC TAC GCA TCT CCA AGC ATA GCT GGC TCA CGG CCT GGT GGC ATC
D W Q G G V Y A S P S I A G S R P G G I
1201/401 1231/411
ATT GCA GCC TGT TGG GCG GCC TTG ATG CAC TTC GGT GAG AAC GGC TAT GTT GAA GCT ACC
I A A C W A A L M H F G E N G Y V E A T
1261/421 1291/431
AAA CAG ATC ATC AAA ACT GCT CGC TTC CTG AAG TCA GAA CTG GAA AAC ATC AAA AAC ATC
K Q I I K T A R F L K S E L E N I K N I
1321/441 1351/451
TTC ATT TTC GGT GAT CCT CAA TTG TCA GTT ATT GCT CTG GGA TCC AAC GAT TTT GAC ATT
F I F G D P Q L S V I A L G S N D F D I
1381/461 1411/471
TAC CGA CTA TCT AAT ATG ATG TCT GCT AAG GGG TGG AAT TTT AAC TAC CTG CAG TTC CCA
Y R L S N M M S A K G W N F N Y L Q F P
1441/481 1471/491
AGA AGC ATT CAT TTC TGC ATT ACG TTA GTA CAT ACT CGG AAG CGA GTG GCG ATC CAG TTC
R S I H F C I T L V H T R K R V A I Q F
1501/501 1531/511
CTA AAG GAT ATC CGG GAA TCA GTC ACA CAA ATC ATG AAG AAT CCT AAA GCT AAG ACC ACA
L K D I R E S V T Q I M K N P K A K T T

Fig. 3C

Mouse S-1-P Lyase Gene -> 1-phase Translation

```
1561/521                                1591/531
GGA ATG GGT GCC ATC TAT GGC ATG GCC CAG GCA ACC ATT GAC AGG AAG CTG GTT GCA GAA
G   M   G   A   I   Y   G   M   A   Q   A   T   I   D   R   K   L   V   A   E
1621/541                                1651/551
ATA TCC TCC GTC TTC TTG GAC TGC CTT TAT ACT ACG GAC CCC GTG ACT CAG GGC AAC CAG
I   S   S   V   F   L   D   C   L   Y   T   T   D   P   V   T   Q   G   N   Q
1681/561
ATG AAC GGT TCT CCA AAG CCC CGC TGA
M   N   G   S   P   K   P   R   *
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Fig. 4A

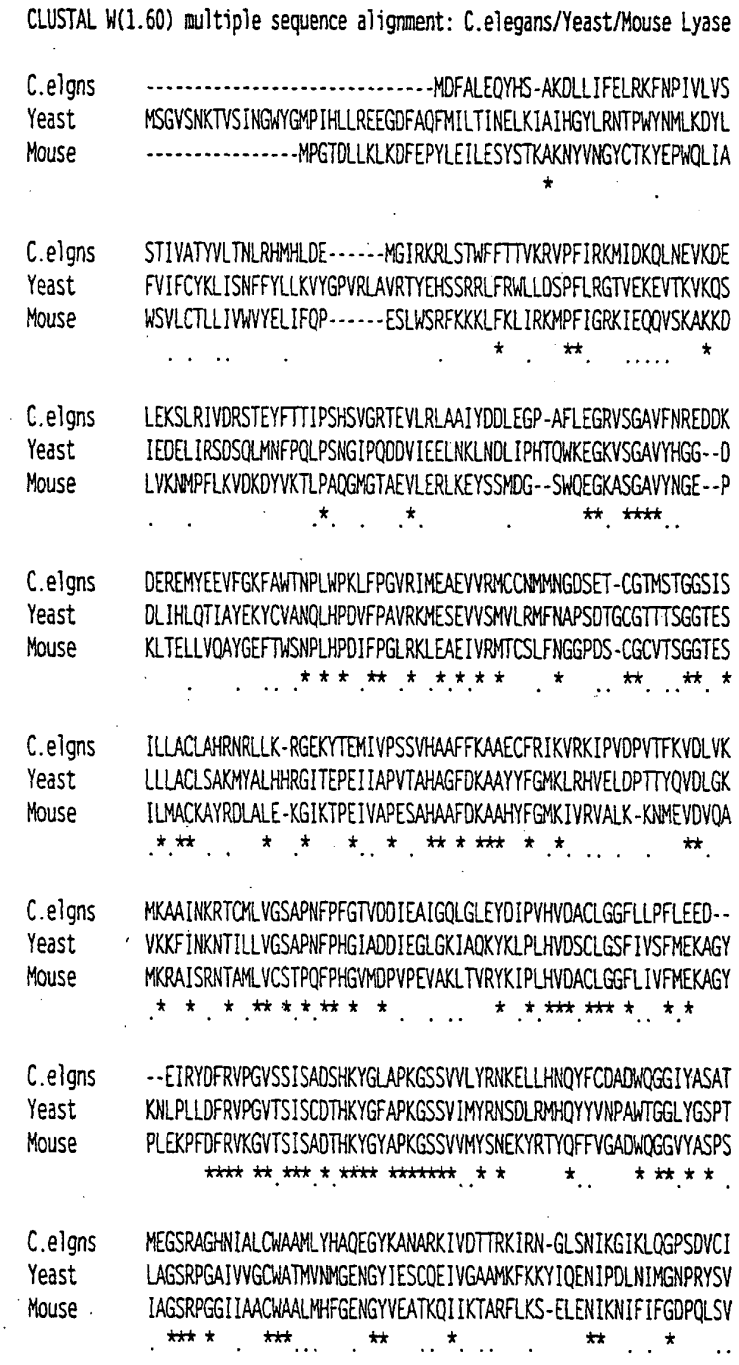

CLUSTAL W(1.60) multiple sequence alignment: C.elegans/Yeast/Mouse Lyase Seq.
C.elgns ----------MDFALEQYHS-AKDLLIFELRKFNPIVLVS
Yeast MSGVSNKTVSINGWYGMPIHLLREEGDFAQFMILTINELKIAIHGYLRNTPWYNMLKDYL
Mouse ----------MPGTDLLKLKDFEPYLEILESYSTKAKNYVNGYCTKYEPWQLIA
C.elgns STIVATYVLTNLRHMHLDE------MGIRKRLSTWFFTTVKRVPFIRKMIDKQLNEVKDE
Yeast FVIFCYKLISNFFYLLKVYGPVRLAVRTYEHSSRRLFRWLLDSPFLRGTVEKEVTKVKQS
Mouse WSVLCTLLIVWVYELIFQP------ESLWSRFKKKLFKLIRKMPFIGRKIEQQVSKAKKD
C.elgns LEKSLRIVDRSTEYFTTIPSHSVGRTEVLRLAAIYDDLEGP-AFLEGRVSGAVFNREDDK
Yeast IEDELIRSDSQLMNFPQLPSNGIPQDDVIEELNKLNDLIPHTQWKEGKVSGAVYHGG--D
Mouse LVKNMPFLKVDKDYVKTLPAQGMGTAEVLERLKEYSSMDG--SWQEGKASGAVYNGE--P
C.elgns DEREMYEEVFGKFAWTNPLWPKLFPGVRIMEAEVVRMCCNMMNGDSET-CGTMSTGGSIS
Yeast DLIHLQTIAYEKYCVANQLHPDVFPAVRKMESEVVSMVLRMFNAPSDTGCGTTTSGGTES
Mouse KLTELLVQAYGEFTWSNPLHPDIFPGLRKLEAEIVRMTCSLFNGGPDS-CGCVTSGGTES
C.elgns ILLACLAHRNRLLK-RGEKYTEMIVPSSVHAAFFKAAECFRIKVRKIPVDPVTFKVDLVK
Yeast LLLACLSAKMYALHHRGITEPEIIAPVTAHAGFDKAAYYFGMKLRHVELDPTTYQVDLGK
Mouse ILMACKAYRDLALE-KGIKTPEIVAPESAHAAFDKAAHYFGMKIVRVALK-KNMEVDVQA
C.elgns MKAAINKRTCMLVGSAPNFPFGTVDDIEAIGQLGLEYDIPVHVDACLGGFLLPFLEED--
Yeast VKKFINKNTILLVGSAPNFPHGIADDIEGLGKIAQKYKLPLHVDSCLGSFIVSFMEKAGY
Mouse MKRAISRNTAMLVCSTPQFPHGVMDPVPEVAKLTVRYKIPLHVDACLGGFLIVFMEKAGY
C.elgns --EIRYDFRVPGVSSISADSHKYGLAPKGSSVVLYRNKELLHNQYFCDADWQGGIYASAT
Yeast KNLPLLDFRVPGVTSISCDTHKYGFAPKGSSVIMYRNSDLRMHQYYVNPAWTGGLYGSPT
Mouse PLEKPFDFRVKGVTSISADTHKYGYAPKGSSVVMYSNEKYRTYQFFVGADWQGGVYASPS
C.elgns MEGSRAGHNIALCWAAMLYHAQEGYKANARKIVDTTRKIRN-GLSNIKGIKLQGPSDVCI
Yeast LAGSRPGAIVVGCWATMVNMGENGYIESCQEIVGAAMKFKKYIQENIPDLNIMGNPRYSV
Mouse IAGSRPGGIIAACWAALMHFGENGYVEATKQIIKTARFLKS-ELENIKNIFIFGDPQLSV

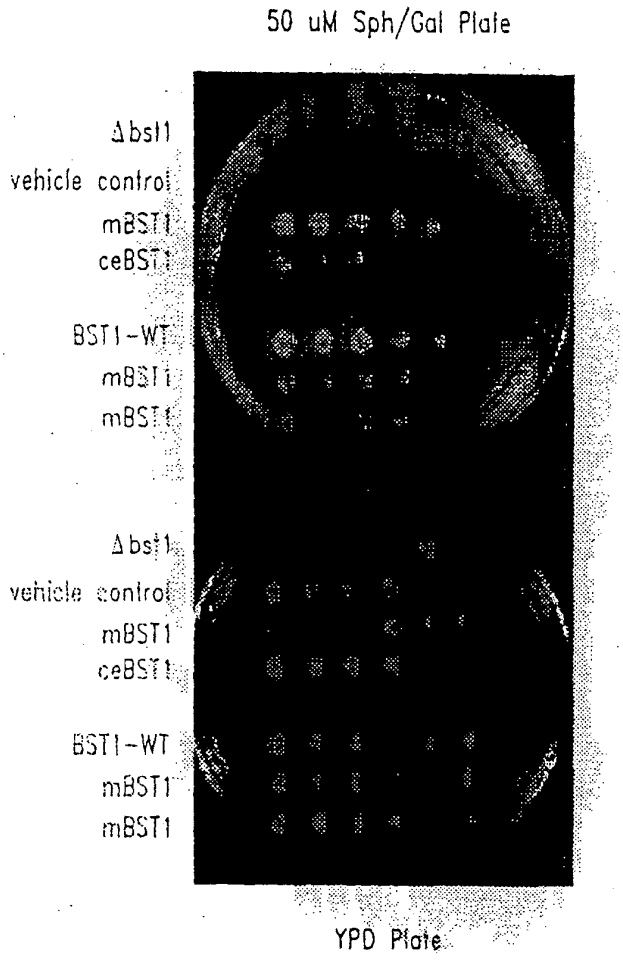
Fig. 4B

```
C.elgns     VSWTTNDGVELYRFHNFMKEKHWQLNGLQFPAGVHIMVTMNHTHG-LAEAFVADCRAAVE
Yeast       ISFSSKT-LNIHELSDRLSKKGWHFNALQKPVALHMAFTRLSAHV--VDEICDILRTTVQ
Mouse       IALGSND-FDIYRLSNMMSAKGWNFNYLQFPRSIHFCITLVHTRKRVAIQFLKDIRESVT
                     .. .   .    . ** * ** *  .*  *  ..         * .*

C.elgns     FVKSHKPSESDKTSEAAIYGLAQSIPDRSLVHEFAHSYIDAVYALTE------------
Yeast       ELKSESNSKPSPDGTSALYGVAGSVKTAGVADKLIVGFLDALYKLGPGEDTATK------
Mouse       QIMKN-P-KAKTTGMGAIYGMAQATIDRKLVAEISSVFLDCLYTTDPVTQGNQMNGSPKP
                      .            *.**.* .    .       ..*..*

C.elgns     -
Yeast       -
Mouse       R
```

Note to the sequence alignment: * = identical residues; . = conserved residues; - = gap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vehicle control
mBST1
ceBST1
BST1-WT
mBST1
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vehicle control
mBST1
ceBST1
BST1-WT
mBST1
mBST1
YPD Plate

Fig. 5

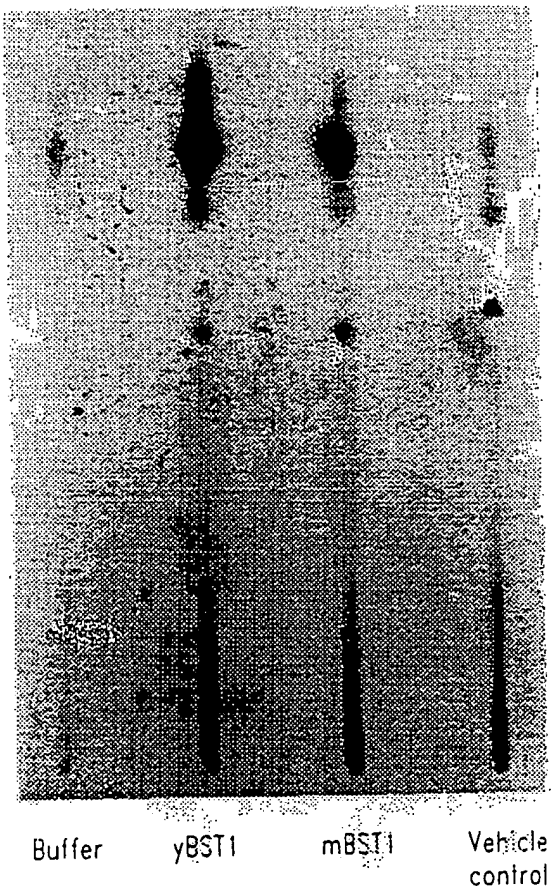
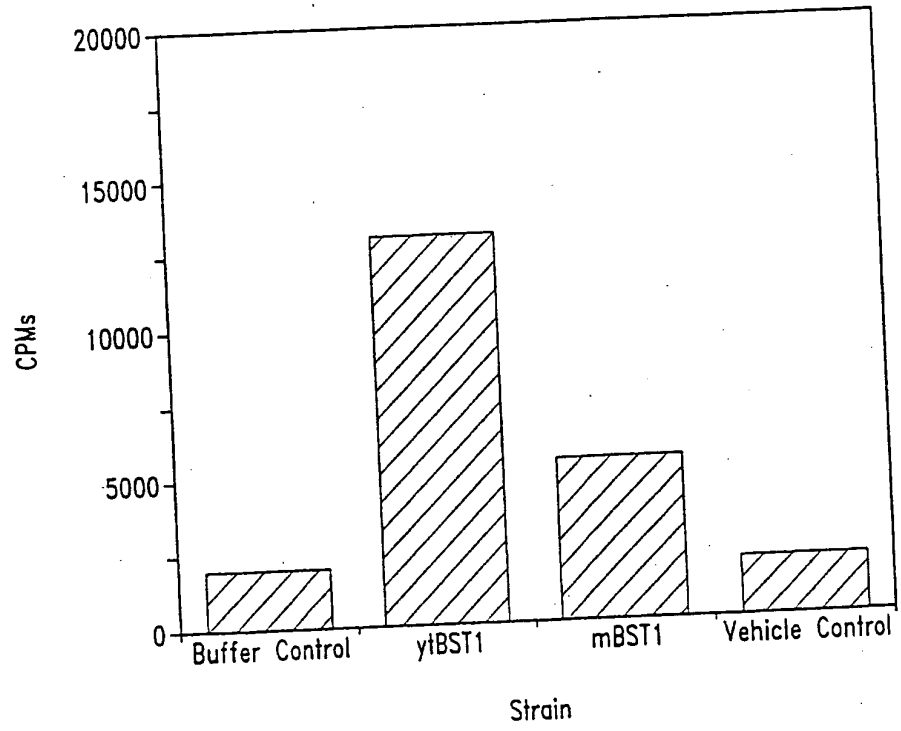


Fig. 6A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10000
5000
0
CPMs
Buffer Control
ytBST1
mBST1
Vehicle Control
Strain

Fig. 6B

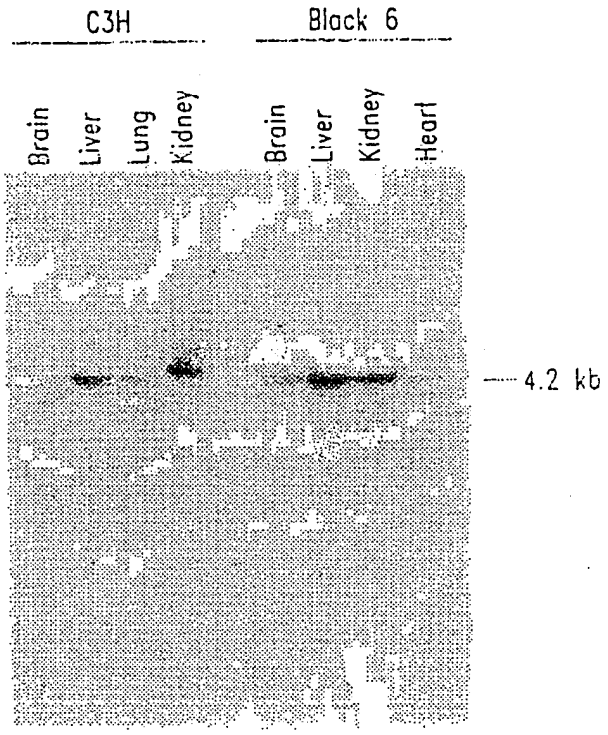
C3H
Black 6
Brain
Liver
Lung
Kidney
Brain
Liver
Kidney
Heart
4.2 kb

Fig. 7

Fig. 8A

```
ATG CCT AGC ACA GAC CTT CTG ATG TTG AAG GCC TTT GAG CCC TAC TTA          48
Met Pro Ser Thr Asp Leu Leu Met Leu Lys Ala Phe Glu Pro Tyr Leu
 1               5                  10                  15

GAG ATT TTG GAA GTA TAC TCC ACA AAA GCC AAG AAT TAT GTA AAT GGA          96
Glu Ile Leu Glu Val Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30

CAT TGC ACC AAG TAT GAG CCC TGG CAG CTA ATT GCA TGG AGT GTC GTG         144
His Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Val
        35                  40                  45

TGG ACC CTG CTG ATA GTC TGG GGA TAT GAG TTT GTC TTC CAG CCA GAG         192
Trp Thr Leu Leu Ile Val Trp Gly Tyr Glu Phe Val Phe Gln Pro Glu
    50                  55                  60

AGT TTA TGG TCA AGG TTT AAA AAG AAA TGT TTT AAG CTC ACC AGG AAG         240
Ser Leu Trp Ser Arg Phe Lys Lys Lys Cys Phe Lys Leu Thr Arg Lys
65                  70                  75                  80

ATG CCC ATT ATT GGT CGT AAG ATT CAA GAC AAG TTG AAC AAG ACC AAG         288
Met Pro Ile Ile Gly Arg Lys Ile Gln Asp Lys Leu Asn Lys Thr Lys
                85                  90                  95

GAT GAT ATT AGC AAG AAC ATG TCA TTC CTG AAA GTG GAC AAA GAG TAT         336
Asp Asp Ile Ser Lys Asn Met Ser Phe Leu Lys Val Asp Lys Glu Tyr
            100                 105                 110

GTG AAA GCT TTA CCC TCC CAG GGT CTG AGC TCA TCT GCT GTT TTG GAG         384
Val Lys Ala Leu Pro Ser Gln Gly Leu Ser Ser Ser Ala Val Leu Glu
        115                 120                 125

AAA CTT AAG GAG TAC AGC TCT ATG GAC GCC TTC TGG CAA GAG GGG AGA         432
Lys Leu Lys Glu Tyr Ser Ser Met Asp Ala Phe Trp Gln Glu Gly Arg
    130                 135                 140

GCC TCT GGA ACA GTG TAC AGT GGG GAG GAG AAG CTC ACT GAG CTC CTT         480
Ala Ser Gly Thr Val Tyr Ser Gly Glu Glu Lys Leu Thr Glu Leu Leu
145                 150                 155                 160
```

Fig. 8B

```
GTG AAG GCT TAT GGA GAT TTT GCA TGG AGT AAC CCC CTG CAT CCA GAT      528
Val Lys Ala Tyr Gly Asp Phe Ala Trp Ser Asn Pro Leu His Pro Asp
                165                 170                 175

ATC TTC CCA GGA CTA CGC AAG ATA GAG GCA GAA ATT GTG AGG ATA GCT      576
Ile Phe Pro Gly Leu Arg Lys Ile Glu Ala Glu Ile Val Arg Ile Ala
            180                 185                 190

TGT TCC CTG TTC AAT GGG GGA CCA GAT TCG TGT GGA TGT GTG ACT TCT      624
Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
        195                 200                 205

GGG GGA ACA GAA AGC ATA CTC ATG GCC TGC AAA GCA TGT CGG GAT CTG      672
Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Cys Arg Asp Leu
    210                 215                 220

GCC TTT GAG AAG GGG ATC AAA ACT CCA GAA ATT GTG GCT CCC CAA AGT      720
Ala Phe Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Gln Ser
225                 230                 235                 240

GCC CAT GCT GCA TTT AAC AAA GCA GCC AGT TAC TTT GGG ATG AAG ATT      768
Ala His Ala Ala Phe Asn Lys Ala Ala Ser Tyr Phe Gly Met Lys Ile
                245                 250                 255

GTG CGG GTC CCA TTG ACG AAG ATG ATG GAG GTG GAT GTG AGG GCA ATG      816
Val Arg Val Pro Leu Thr Lys Met Met Glu Val Asp Val Arg Ala Met
            260                 265                 270

AGA AGA GCT ATC TCC AGG AAC ACT GCC ATG CTC GTC TGT TCT ACC CCA      864
Arg Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
        275                 280                 285

CAG TTT CCT CAT GGT GTA ATA GAT CCT GTC CCT GAA GTG GCC AAG CTG      912
Gln Phe Pro His Gly Val Ile Asp Pro Val Pro Glu Val Ala Lys Leu
    290                 295                 300

GCT GTC AAA TAC AAA ATA CCC CTT CAT GTC GAC GCT TGT CTG GGA GGC      960
Ala Val Lys Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305                 310                 315                 320
```

Fig. 8C

```
TTC CTC ATC GTC TTT ATG GAG AAA GCA GGA TAC CCA CTG GAG CAC CCA      1008
Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu His Pro
            325                 330                 335

TTT GAT TTC CGG GTG AAA GGT GTA ACC AGC ATT TCA GCT GAC ACC CAT      1056
Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
        340                 345                 350

AAG TAT GGC TAT GCC CCA AAA GGC TCA TCA TTG GTG TTG TAT AGT GAC      1104
Lys Tyr Gly Tyr Ala Pro Lys Gly Ser Ser Leu Val Leu Tyr Ser Asp
    355                 360                 365

AAG AAG TAC AGG AAC TAT CAG TTC TTC GTC GAT ACA GAT TGG CAG GGT      1152
Lys Lys Tyr Arg Asn Tyr Gln Phe Phe Val Asp Thr Asp Trp Gln Gly
370                 375                 380

GGC ATC TAT GCT TCC CCA ACC ATC GCA GGC TCA CGG CCT GGT GGC ATT      1200
Gly Ile Tyr Ala Ser Pro Thr Ile Ala Gly Ser Arg Pro Gly Gly Ile
385                 390                 395                 400

AGC GCA GCC TGT TGG GCT GCC TTG ATG CAC TTC GGT GAG AAC GGC TAT      1248
Ser Ala Ala Cys Trp Ala Ala Leu Met His Phe Gly Glu Asn Gly Tyr
            405                 410                 415

GTT GAA GCT ACC AAA CAG ATC ATC AAA ACT GCT CGC TTC CTC AAG TCA      1296
Val Glu Ala Thr Lys Gln Ile Ile Lys Thr Ala Arg Phe Leu Lys Ser
        420                 425                 430

GAA CTG GAA AAT ATC AAA GGC ATC TTT GTT TTT GGG AAT CCC CAA TTG      1344
Glu Leu Glu Asn Ile Lys Gly Ile Phe Val Phe Gly Asn Pro Gln Leu
    435                 440                 445

TCA CTC ATT GCT CTG GGA TCC CGT GAT TTT GAC ATC TAC CGA CTA TCA      1392
Ser Leu Ile Ala Leu Gly Ser Arg Asp Phe Asp Ile Tyr Arg Leu Ser
450                 455                 460

AAC CTG ATG ACT GCT AAG GGG TGG AAC TTG AAC CAG TTG CAG TTC CCA      1440
Asn Leu Met Thr Ala Lys Gly Trp Asn Leu Asn Gln Leu Gln Phe Pro
465                 470                 475                 480
```

Fig. 8D

```
CCC AGT ATT CAT TTC TGC ATC ACA TTA CTA CAC GCC CGG AAA CGA GTA      1488
Pro Ser Ile His Phe Cys Ile Thr Leu Leu His Ala Arg Lys Arg Val
                485                 490                 495

GCT ATA CAA TTC CTA AAG GAC ATT CGA GAA TCT GTC ACT CAA ATC ATG      1536
Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
            500                 505                 510

AAG AAT CCT AAA GCG AAG ACC ACA GGA ATG GGT GCC ATC TAT GCC ATG      1584
Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Ala Met
        515                 520                 525

GCC CAG ACA ACT GTT GAC AGG AAT ATG GTT GCA GAA TTG TCC TCA GTC      1632
Ala Gln Thr Thr Val Asp Arg Asn Met Val Ala Glu Leu Ser Ser Val
    530                 535                 540

TTC TTG GAC AGC TTG TAC AGC ACC GAC ACT GTC ACC CAG GGC AGC CAG      1680
Phe Leu Asp Ser Leu Tyr Ser Thr Asp Thr Val Thr Gln Gly Ser Gln
545                 550                 555                 560

ATG AAT GGT TCT CCA AAA CCC CAC TGA                                  1707
Met Asn Gly Ser Pro Lys Pro His
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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Saba, Julie D.
Zhou, Jianhui

(ii) TITLE OF INVENTION: SPHINGOSINE-1-PHOSPHATE LYASE POLYPEPTIDES, POLYNUCLEOTIDES AND MODULATING AGENTS AND METHODS OF USE THEREFOR

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SEED and BERRY LLP
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 29-SEP-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: David, Maki J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 200116.402

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 622-4900
(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1707 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1704

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAG ATT TTG GAA TCT TAT TCC ACA AAA GCC AAG AAT TAT GTG AAT GGA      96
Glu Ile Leu Glu Ser Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30

TAT TGC ACC AAA TAT GAG CCC TGG CAG CTC ATT GCG TGG AGT GTC CTG     144
Tyr Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Leu
        35                  40                  45

TGT ACT CTG CTG ATA GTC TGG GTG TAT GAG CTT ATC TTC CAG CCA GAG     192
Cys Thr Leu Leu Ile Val Trp Val Tyr Glu Leu Ile Phe Gln Pro Glu
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AGT TTA TGG TCT CGG TTT AAA AAA AAA TTA TTT AAG CTT ATC AGG AAG 240
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65 70 75 80

ATG CCA TTT ATT GGA CGT AAG ATC GAA CAA CAG GTG AGC AAA GCC AAG 288
Met Pro Phe Ile Gly Arg Lys Ile Glu Gln Gln Val Ser Lys Ala Lys
85 90 95

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Lys Asp Leu Val Lys Asn Met Pro Phe Leu Lys Val Asp Lys Asp Tyr
100 105 110

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115 120 125

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Arg Leu Lys Glu Tyr Ser Ser Met Asp Gly Ser Trp Gln Glu Gly Lys
130 135 140

GCC TCA GGA GCT GTG TAC AAT GGG GAA CCG AAG CTC ACG GAG CTG CTG 480
Ala Ser Gly Ala Val Tyr Asn Gly Glu Pro Lys Leu Thr Glu Leu Leu
145 150 155 160

GTG CAG GCT TAT GGA GAA TTC ACG TGG AGC AAT CCA CTG CAT CCA GAT 528
Val Gln Ala Tyr Gly Glu Phe Thr Trp Ser Asn Pro Leu His Pro Asp
165 170 175

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Ile Phe Pro Gly Leu Arg Lys Leu Glu Ala Glu Ile Val Arg Met Thr
180 185 190

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Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
195 200 205

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Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Tyr Arg Asp Leu
    210                 215                 220

GCG TTA GAG AAG GGG ATC AAA ACT CCA GAA ATT GTG GCT CCC GAG AGT       720
Ala Leu Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Glu Ser
225                 230                 235                 240

GCC CAT GCT GCA TTC GAC AAA GCA GCT CAT TAT TTT GGG ATG AAG ATT       768
Ala His Ala Ala Phe Asp Lys Ala Ala His Tyr Phe Gly Met Lys Ile
                245                 250                 255

GTC CGA GTT GCA CTG AAA AAG AAC ATG GAG GTG GAT GTG CAG GCA ATG       816
Val Arg Val Ala Leu Lys Lys Asn Met Glu Val Asp Val Gln Ala Met
            260                 265                 270

AAG AGA GCC ATC TCC AGG AAC ACA GCT ATG CTG GTC TGT TCT ACC CCA       864
Lys Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
        275                 280                 285

CAG TTT CCT CAT GGT GTG ATG GAT CCT GTC CCC GAA GTG GCC AAG TTA       912
Gln Phe Pro His Gly Val Met Asp Pro Val Pro Glu Val Ala Lys Leu
    290                 295                 300

ACT GTC AGA TAT AAA ATC CCA CTC CAT GTG GAT GCT TGT CTG GGG GGC       960
Thr Val Arg Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305                 310                 315                 320

TTC CTC ATT GTC TTC ATG GAG AAA GCA GGG TAC CCA CTG GAG AAA CCA      1008
Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu Lys Pro
                325                 330                 335

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            340                 345                 350
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GTT GAA GCT ACC AAA CAG ATC ATC AAA ACT GCT CGC TTC CTG AAG TCA 1296
Val Glu Ala Thr Lys Gln Ile Ile Lys Thr Ala Arg Phe Leu Lys Ser
420 425 430

GAA CTG GAA AAC ATC AAA AAC ATC TTC ATT TTC GGT GAT CCT CAA TTG 1344
Glu Leu Glu Asn Ile Lys Asn Ile Phe Ile Phe Gly Asp Pro Gln Leu
435 440 445

TCA GTT ATT GCT CTG GGA TCC AAC GAT TTT GAC ATT TAC CGA CTA TCT 1392
Ser Val Ile Ala Leu Gly Ser Asn Asp Phe Asp Ile Tyr Arg Leu Ser
450 455 460

AAT ATG ATG TCT GCT AAG GGG TGG AAT TTT AAC TAC CTG CAG TTC CCA 1440
Asn Met Met Ser Ala Lys Gly Trp Asn Phe Asn Tyr Leu Gln Phe Pro
465 470 475 480

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Arg Ser Ile His Phe Cys Ile Thr Leu Val His Thr Arg Lys Arg Val
485 490 495

GCG ATC CAG TTC CTA AAG GAT ATC CGG GAA TCA GTC ACA CAA ATC ATG 1536

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Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
            500                 505                 510

AAG AAT CCT AAA GCT AAG ACC ACA GGA ATG GGT GCC ATC TAT GGC ATG      1584
Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Gly Met
        515                 520                 525

GCC CAG GCA ACC ATT GAC AGG AAG CTG GTT GCA GAA ATA TCC TCC GTC      1632
Ala Gln Ala Thr Ile Asp Arg Lys Leu Val Ala Glu Ile Ser Ser Val
    530                 535                 540

TTC TTG GAC TGC CTT TAT ACT ACG GAC CCC GTG ACT CAG GGC AAC CAG      1680
Phe Leu Asp Cys Leu Tyr Thr Thr Asp Pro Val Thr Gln Gly Asn Gln
545                 550                 555                 560

ATG AAC GGT TCT CCA AAG CCC CGC TGA                                  1707
Met Asn Gly Ser Pro Lys Pro Arg
                565
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 568 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Gly Thr Asp Leu Leu Lys Leu Lys Asp Phe Glu Pro Tyr Leu
 1               5                  10                  15

Glu Ile Leu Glu Ser Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30
```

Tyr Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Leu
35 40 45

Cys Thr Leu Leu Ile Val Trp Val Tyr Glu Leu Ile Phe Gln Pro Glu
50 55 60

Ser Leu Trp Ser Arg Phe Lys Lys Lys Leu Phe Lys Leu Ile Arg Lys
65 70 75 80

Met Pro Phe Ile Gly Arg Lys Ile Glu Gln Gln Val Ser Lys Ala Lys
85 90 95

Lys Asp Leu Val Lys Asn Met Pro Phe Leu Lys Val Asp Lys Asp Tyr
100 105 110

Val Lys Thr Leu Pro Ala Gln Gly Met Gly Thr Ala Glu Val Leu Glu
115 120 125

Arg Leu Lys Glu Tyr Ser Ser Met Asp Gly Ser Trp Gln Glu Gly Lys
130 135 140

Ala Ser Gly Ala Val Tyr Asn Gly Glu Pro Lys Leu Thr Glu Leu Leu
145 150 155 160

Val Gln Ala Tyr Gly Glu Phe Thr Trp Ser Asn Pro Leu His Pro Asp
165 170 175

Ile Phe Pro Gly Leu Arg Lys Leu Glu Ala Glu Ile Val Arg Met Thr
180 185 190

Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
195 200 205

Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Tyr Arg Asp Leu
210 215 220

Ala Leu Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Glu Ser

225 230 235 240

Ala His Ala Ala Phe Asp Lys Ala Ala His Tyr Phe Gly Met Lys Ile
245 250 255

Val Arg Val Ala Leu Lys Lys Asn Met Glu Val Asp Val Gln Ala Met
260 265 270

Lys Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
275 280 285

Gln Phe Pro His Gly Val Met Asp Pro Val Pro Glu Val Ala Lys Leu
290 295 300

Thr Val Arg Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305 310 315 320

Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu Lys Pro
325 330 335

Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
340 345 350

Lys Tyr Gly Tyr Ala Pro Lys Gly Ser Ser Val Val Met Tyr Ser Asn
355 360 365

Glu Lys Tyr Arg Thr Tyr Gln Phe Phe Val Gly Ala Asp Trp Gln Gly
370 375 380

Gly Val Tyr Ala Ser Pro Ser Ile Ala Gly Ser Arg Pro Gly Gly Ile
385 390 395 400

Ile Ala Ala Cys Trp Ala Ala Leu Met His Phe Gly Glu Asn Gly Tyr
405 410 415

Val Glu Ala Thr Lys Gln Ile Ile Lys Thr Ala Arg Phe Leu Lys Ser
420 425 430

Glu Leu Glu Asn Ile Lys Asn Ile Phe Ile Phe Gly Asp Pro Gln Leu
435 440 445

Ser Val Ile Ala Leu Gly Ser Asn Asp Phe Asp Ile Tyr Arg Leu Ser
450 455 460

Asn Met Met Ser Ala Lys Gly Trp Asn Phe Asn Tyr Leu Gln Phe Pro
465 470 475 480

Arg Ser Ile His Phe Cys Ile Thr Leu Val His Thr Arg Lys Arg Val
485 490 495

Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
500 505 510

Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Gly Met
515 520 525

Ala Gln Ala Thr Ile Asp Arg Lys Leu Val Ala Glu Ile Ser Ser Val
530 535 540

Phe Leu Asp Cys Leu Tyr Thr Thr Asp Pro Val Thr Gln Gly Asn Gln
545 550 555 560

Met Asn Gly Ser Pro Lys Pro Arg
565

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1707 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1704

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG CCT AGC ACA GAC CTT CTG ATG TTG AAG GCC TTT GAG CCC TAC TTA        48
Met Pro Ser Thr Asp Leu Leu Met Leu Lys Ala Phe Glu Pro Tyr Leu
 1               5                  10                  15

GAG ATT TTG GAA GTA TAC TCC ACA AAA GCC AAG AAT TAT GTA AAT GGA        96
Glu Ile Leu Glu Val Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30

CAT TGC ACC AAG TAT GAG CCC TGG CAG CTA ATT GCA TGG AGT GTC GTG       144
His Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Val
        35                  40                  45

TGG ACC CTG CTG ATA GTC TGG GGA TAT GAG TTT GTC TTC CAG CCA GAG       192
Trp Thr Leu Leu Ile Val Trp Gly Tyr Glu Phe Val Phe Gln Pro Glu
    50                  55                  60

AGT TTA TGG TCA AGG TTT AAA AAG AAA TGT TTT AAG CTC ACC AGG AAG       240
Ser Leu Trp Ser Arg Phe Lys Lys Lys Cys Phe Lys Leu Thr Arg Lys
65                  70                  75                  80

ATG CCC ATT ATT GGT CGT AAG ATT CAA GAC AAG TTG AAC AAG ACC AAG       288
Met Pro Ile Ile Gly Arg Lys Ile Gln Asp Lys Leu Asn Lys Thr Lys
                85                  90                  95

GAT GAT ATT AGC AAG AAC ATG TCA TTC CTG AAA GTG GAC AAA GAG TAT       336
Asp Asp Ile Ser Lys Asn Met Ser Phe Leu Lys Val Asp Lys Glu Tyr
            100                 105                 110

GTG AAA GCT TTA CCC TCC CAG GGT CTG AGC TCA TCT GCT GTT TTG GAG       384
```

Val Lys Ala Leu Pro Ser Gln Gly Leu Ser Ser Ser Ala Val Leu Glu
115 120 125

AAA CTT AAG GAG TAC AGC TCT ATG GAC GCC TTC TGG CAA GAG GGG AGA 432
Lys Leu Lys Glu Tyr Ser Ser Met Asp Ala Phe Trp Gln Glu Gly Arg
130 135 140

GCC TCT GGA ACA GTG TAC AGT GGG GAG GAG AAG CTC ACT GAG CTC CTT 480
Ala Ser Gly Thr Val Tyr Ser Gly Glu Glu Lys Leu Thr Glu Leu Leu
145 150 155 160

GTG AAG GCT TAT GGA GAT TTT GCA TGG AGT AAC CCC CTG CAT CCA GAT 528
Val Lys Ala Tyr Gly Asp Phe Ala Trp Ser Asn Pro Leu His Pro Asp
165 170 175

ATC TTC CCA GGA CTA CGC AAG ATA GAG GCA GAA ATT GTG AGG ATA GCT 576
Ile Phe Pro Gly Leu Arg Lys Ile Glu Ala Glu Ile Val Arg Ile Ala
180 185 190

TGT TCC CTG TTC AAT GGG GGA CCA GAT TCG TGT GGA TGT GTG ACT TCT 624
Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
195 200 205

GGG GGA ACA GAA AGC ATA CTC ATG GCC TGC AAA GCA TGT CGG GAT CTG 672
Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Cys Arg Asp Leu
210 215 220

GCC TTT GAG AAG GGG ATC AAA ACT CCA GAA ATT GTG GCT CCC CAA AGT 720
Ala Phe Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Gln Ser
225 230 235 240

GCC CAT GCT GCA TTT AAC AAA GCA GCC AGT TAC TTT GGG ATG AAG ATT 768
Ala His Ala Ala Phe Asn Lys Ala Ala Ser Tyr Phe Gly Met Lys Ile
245 250 255

GTG CGG GTC CCA TTG ACG AAG ATG ATG GAG GTG GAT GTG AGG GCA ATG 816
Val Arg Val Pro Leu Thr Lys Met Met Glu Val Asp Val Arg Ala Met

```
            260                 265                 270

AGA AGA GCT ATC TCC AGG AAC ACT GCC ATG CTC GTC TGT TCT ACC CCA      864
Arg Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
        275                 280                 285

CAG TTT CCT CAT GGT GTA ATA GAT CCT GTC CCT GAA GTG GCC AAG CTG      912
Gln Phe Pro His Gly Val Ile Asp Pro Val Pro Glu Val Ala Lys Leu
    290                 295                 300

GCT GTC AAA TAC AAA ATA CCC CTT CAT GTC GAC GCT TGT CTG GGA GGC      960
Ala Val Lys Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305                 310                 315                 320

TTC CTC ATC GTC TTT ATG GAG AAA GCA GGA TAC CCA CTG GAG CAC CCA     1008
Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu His Pro
                325                 330                 335

TTT GAT TTC CGG GTG AAA GGT GTA ACC AGC ATT TCA GCT GAC ACC CAT     1056
Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
            340                 345                 350

AAG TAT GGC TAT GCC CCA AAA GGC TCA TCA TTG GTG TTG TAT AGT GAC     1104
Lys Tyr Gly Tyr Ala Pro Lys Gly Ser Ser Leu Val Leu Tyr Ser Asp
        355                 360                 365

AAG AAG TAC AGG AAC TAT CAG TTC TTC GTC GAT ACA GAT TGG CAG GGT     1152
Lys Lys Tyr Arg Asn Tyr Gln Phe Phe Val Asp Thr Asp Trp Gln Gly
    370                 375                 380

GGC ATC TAT GCT TCC CCA ACC ATC GCA GGC TCA CGG CCT GGT GGC ATT     1200
Gly Ile Tyr Ala Ser Pro Thr Ile Ala Gly Ser Arg Pro Gly Gly Ile
385                 390                 395                 400

AGC GCA GCC TGT TGG GCT GCC TTG ATG CAC TTC GGT GAG AAC GGC TAT     1248
Ser Ala Ala Cys Trp Ala Ala Leu Met His Phe Gly Glu Asn Gly Tyr
                405                 410                 415
```

GTT GAA GCT ACC AAA CAG ATC ATC AAA ACT GCT CGC TTC CTC AAG TCA 1296
Val Glu Ala Thr Lys Gln Ile Ile Lys Thr Ala Arg Phe Leu Lys Ser
420 425 430

GAA CTG GAA AAT ATC AAA GGC ATC TTT GTT TTT GGG AAT CCC CAA TTG 1344
Glu Leu Glu Asn Ile Lys Gly Ile Phe Val Phe Gly Asn Pro Gln Leu
435 440 445

TCA CTC ATT GCT CTG GGA TCC CGT GAT TTT GAC ATC TAC CGA CTA TCA 1392
Ser Leu Ile Ala Leu Gly Ser Arg Asp Phe Asp Ile Tyr Arg Leu Ser
450 455 460

AAC CTG ATG ACT GCT AAG GGG TGG AAC TTG AAC CAG TTG CAG TTC CCA 1440
Asn Leu Met Thr Ala Lys Gly Trp Asn Leu Asn Gln Leu Gln Phe Pro
465 470 475 480

CCC AGT ATT CAT TTC TGC ATC ACA TTA CTA CAC GCC CGG AAA CGA GTA 1488
Pro Ser Ile His Phe Cys Ile Thr Leu Leu His Ala Arg Lys Arg Val
485 490 495

GCT ATA CAA TTC CTA AAG GAC ATT CGA GAA TCT GTC ACT CAA ATC ATG 1536
Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
500 505 510

AAG AAT CCT AAA GCG AAG ACC ACA GGA ATG GGT GCC ATC TAT GCC ATG 1584
Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Ala Met
515 520 525

GCC CAG ACA ACT GTT GAC AGG AAT ATG GTT GCA GAA TTG TCC TCA GTC 1632
Ala Gln Thr Thr Val Asp Arg Asn Met Val Ala Glu Leu Ser Ser Val
530 535 540

TTC TTG GAC AGC TTG TAC AGC ACC GAC ACT GTC ACC CAG GGC AGC CAG 1680
Phe Leu Asp Ser Leu Tyr Ser Thr Asp Thr Val Thr Gln Gly Ser Gln
545 550 555 560

```
ATG AAT GGT TCT CCA AAA CCC CAC TGA                                  1707
Met Asn Gly Ser Pro Lys Pro His
                565
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 568 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Pro Ser Thr Asp Leu Leu Met Leu Lys Ala Phe Glu Pro Tyr Leu
 1               5                  10                  15

Glu Ile Leu Glu Val Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30

His Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Val
        35                  40                  45

Trp Thr Leu Leu Ile Val Trp Gly Tyr Glu Phe Val Phe Gln Pro Glu
    50                  55                  60

Ser Leu Trp Ser Arg Phe Lys Lys Lys Cys Phe Lys Leu Thr Arg Lys
65                  70                  75                  80

Met Pro Ile Ile Gly Arg Lys Ile Gln Asp Lys Leu Asn Lys Thr Lys
                85                  90                  95

Asp Asp Ile Ser Lys Asn Met Ser Phe Leu Lys Val Asp Lys Glu Tyr
            100                 105                 110
```

Val Lys Ala Leu Pro Ser Gln Gly Leu Ser Ser Ser Ala Val Leu Glu
115 120 125

Lys Leu Lys Glu Tyr Ser Ser Met Asp Ala Phe Trp Gln Glu Gly Arg
130 135 140

Ala Ser Gly Thr Val Tyr Ser Gly Glu Glu Lys Leu Thr Glu Leu Leu
145 150 155 160

Val Lys Ala Tyr Gly Asp Phe Ala Trp Ser Asn Pro Leu His Pro Asp
165 170 175

Ile Phe Pro Gly Leu Arg Lys Ile Glu Ala Glu Ile Val Arg Ile Ala
180 185 190

Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
195 200 205

Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Cys Arg Asp Leu
210 215 220

Ala Phe Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Gln Ser
225 230 235 240

Ala His Ala Ala Phe Asn Lys Ala Ala Ser Tyr Phe Gly Met Lys Ile
245 250 255

Val Arg Val Pro Leu Thr Lys Met Met Glu Val Asp Val Arg Ala Met
260 265 270

Arg Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
275 280 285

Gln Phe Pro His Gly Val Ile Asp Pro Val Pro Glu Val Ala Lys Leu
290 295 300

Ala Val Lys Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly

305 310 315 320

Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu His Pro
325 330 335

Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
340 345 350

Lys Tyr Gly Tyr Ala Pro Lys Gly Ser Ser Leu Val Leu Tyr Ser Asp
355 360 365

Lys Lys Tyr Arg Asn Tyr Gln Phe Phe Val Asp Thr Asp Trp Gln Gly
370 375 380

Gly Ile Tyr Ala Ser Pro Thr Ile Ala Gly Ser Arg Pro Gly Gly Ile
385 390 395 400

Ser Ala Ala Cys Trp Ala Ala Leu Met His Phe Gly Glu Asn Gly Tyr
405 410 415

Val Glu Ala Thr Lys Gln Ile Ile Lys Thr Ala Arg Phe Leu Lys Ser
420 425 430

Glu Leu Glu Asn Ile Lys Gly Ile Phe Val Phe Gly Asn Pro Gln Leu
435 440 445

Ser Leu Ile Ala Leu Gly Ser Arg Asp Phe Asp Ile Tyr Arg Leu Ser
450 455 460

Asn Leu Met Thr Ala Lys Gly Trp Asn Leu Asn Gln Leu Gln Phe Pro
465 470 475 480

Pro Ser Ile His Phe Cys Ile Thr Leu Leu His Ala Arg Lys Arg Val
485 490 495

Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
500 505 510

Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Ala Met
515 520 525

Ala Gln Thr Thr Val Asp Arg Asn Met Val Ala Glu Leu Ser Ser Val
530 535 540

Phe Leu Asp Ser Leu Tyr Ser Thr Asp Thr Val Thr Gln Gly Ser Gln
545 550 555 560

Met Asn Gly Ser Pro Lys Pro His
565

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1626

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GAT TTT GCA CTG GAG CAA TAT CAT AGT GCA AAG GAT TTG TTA ATA 48
Met Asp Phe Ala Leu Glu Gln Tyr His Ser Ala Lys Asp Leu Leu Ile
1 5 10 15

TTT GAG CTT CGA AAG TTC AAT CCA ATT GTT CTG GTT TCT AGT ACT ATT 96
Phe Glu Leu Arg Lys Phe Asn Pro Ile Val Leu Val Ser Ser Thr Ile
20 25 30

```
GTT GCA ACA TAC GTA CTC ACC AAT CTG AGA CAT ATG CAT TTA GAT GAA      144
Val Ala Thr Tyr Val Leu Thr Asn Leu Arg His Met His Leu Asp Glu
         35                  40                  45

ATG GGC ATC CGG AAA CGT TTG AGC ACT TGG TTT TTC ACC ACT GTA AAG      192
Met Gly Ile Arg Lys Arg Leu Ser Thr Trp Phe Phe Thr Thr Val Lys
     50                  55                  60

CGT GTG CCT TTC ATC AGG AAA ATG ATT GAC AAA CAA CTA AAC GAA GTA      240
Arg Val Pro Phe Ile Arg Lys Met Ile Asp Lys Gln Leu Asn Glu Val
 65                  70                  75                  80

AAG GAC GAG CTT GAG AAA AGT CTG AGA ATT GTG GAT CGA AGC ACC GAA      288
Lys Asp Glu Leu Glu Lys Ser Leu Arg Ile Val Asp Arg Ser Thr Glu
                 85                  90                  95

TAC TTC ACT ACA ATC CCA AGC CAT TCA GTT GGA AGA ACT GAA GTA CTT      336
Tyr Phe Thr Thr Ile Pro Ser His Ser Val Gly Arg Thr Glu Val Leu
            100                 105                 110

CGC CTT GCT GCC ATC TAT GAT GAT TTG GAA GGA CCA GCT TTT TTG GAA      384
Arg Leu Ala Ala Ile Tyr Asp Asp Leu Glu Gly Pro Ala Phe Leu Glu
        115                 120                 125

GGA AGA GTA TCT GGA GCA GTC TTC AAT AGA GAA GAC GAC AAG GAC GAA      432
Gly Arg Val Ser Gly Ala Val Phe Asn Arg Glu Asp Asp Lys Asp Glu
    130                 135                 140

CGG GAG ATG TAT GAG GAG GTG TTC GGA AAA TTT GCC TGG ACC AAC CCA      480
Arg Glu Met Tyr Glu Glu Val Phe Gly Lys Phe Ala Trp Thr Asn Pro
145                 150                 155                 160

CTT TGG CCA AAA TTG TTC CCT GGA GTG AGA ATC ATG GAG GCT GAA GTT      528
Leu Trp Pro Lys Leu Phe Pro Gly Val Arg Ile Met Glu Ala Glu Val
                165                 170                 175
```

```
GTT CGC ATG TGT TGT AAT ATG ATG AAT GGA GAT TCG GAG ACA TGT GGA      576
Val Arg Met Cys Cys Asn Met Met Asn Gly Asp Ser Glu Thr Cys Gly
            180                 185                 190

ACT ATG TCA ACT GGT GGA TCC ATT TCA ATT CTT TTG GCG TGC CTG GCT      624
Thr Met Ser Thr Gly Gly Ser Ile Ser Ile Leu Leu Ala Cys Leu Ala
        195                 200                 205

CAT CGT AAT CGT CTT TTG AAA AGA GGA GAA AAG TAC ACA GAG ATG ATT      672
His Arg Asn Arg Leu Leu Lys Arg Gly Glu Lys Tyr Thr Glu Met Ile
    210                 215                 220

GTC CCA TCA TCC GTC CAT GCA GCG TTC TTC AAA GCT GCC GAA TGT TTC      720
Val Pro Ser Ser Val His Ala Ala Phe Phe Lys Ala Ala Glu Cys Phe
225                 230                 235                 240

CGT ATC AAA GTT CGC AAG ATT CCA GTT GAT CCT GTT ACT TTC AAA GTA      768
Arg Ile Lys Val Arg Lys Ile Pro Val Asp Pro Val Thr Phe Lys Val
                245                 250                 255

GAC CTT GTC AAA ATG AAA GCC GCA ATT AAC AAG AGA ACA TGT ATG TTA      816
Asp Leu Val Lys Met Lys Ala Ala Ile Asn Lys Arg Thr Cys Met Leu
            260                 265                 270

GTT GGA TCT GCT CCA AAC TTT CCA TTT GGA ACT GTT GAT GAC ATT GAA      864
Val Gly Ser Ala Pro Asn Phe Pro Phe Gly Thr Val Asp Asp Ile Glu
        275                 280                 285

GCT ATT GGA CAG CTA GGA CTT GAA TAT GAC ATC CCA GTT CAT GTT GAT      912
Ala Ile Gly Gln Leu Gly Leu Glu Tyr Asp Ile Pro Val His Val Asp
    290                 295                 300

GCT TGT CTT GGT GGT TTC CTT CTT CCA TTC CTT GAA GAA GAC GAG ATT      960
Ala Cys Leu Gly Gly Phe Leu Leu Pro Phe Leu Glu Glu Asp Glu Ile
305                 310                 315                 320

CGC TAT GAC TTC CGT GTT CCT GGT GTA TCT TCG ATT TCT GCA GAT AGT     1008
```

```
Arg Tyr Asp Phe Arg Val Pro Gly Val Ser Ser Ile Ser Ala Asp Ser
                325                 330                 335

CAC AAA TAC GGA CTC GCT CCA AAG GGG TCA TCA GTT GTT CTT TAT CGC    1056
His Lys Tyr Gly Leu Ala Pro Lys Gly Ser Ser Val Val Leu Tyr Arg
            340                 345                 350

AAT AAG GAA CTT CTT CAT AAT CAG TAC TTC TGT GAT GCT GAT TGG CAA    1104
Asn Lys Glu Leu Leu His Asn Gln Tyr Phe Cys Asp Ala Asp Trp Gln
        355                 360                 365

GGA GGT ATC TAT GCA TCG GCT ACT ATG GAA GGA TCA CGC GCT GGG CAC    1152
Gly Gly Ile Tyr Ala Ser Ala Thr Met Glu Gly Ser Arg Ala Gly His
    370                 375                 380

AAC ATT GCA CTT TGC TGG GCC GCA ATG CTT TAT CAC GCT CAG GAA GGA    1200
Asn Ile Ala Leu Cys Trp Ala Ala Met Leu Tyr His Ala Gln Glu Gly
385                 390                 395                 400

TAC AAG GCC AAT GCT AGA AAG ATT GTT GAC ACT ACA AGA AAG ATT AGA    1248
Tyr Lys Ala Asn Ala Arg Lys Ile Val Asp Thr Thr Arg Lys Ile Arg
                405                 410                 415

AAT GGA CTT TCA AAC ATT AAG GGA ATC AAA TTA CAA GGG CCA AGT GAT    1296
Asn Gly Leu Ser Asn Ile Lys Gly Ile Lys Leu Gln Gly Pro Ser Asp
            420                 425                 430

GTT TGT ATT GTT AGC TGG ACA ACC AAT GAT GGA GTT GAA CTC TAC AGA    1344
Val Cys Ile Val Ser Trp Thr Thr Asn Asp Gly Val Glu Leu Tyr Arg
        435                 440                 445

TTC CAT AAC TTC ATG AAG GAA AAA CAT TGG CAA CTG AAT GGA CTT CAA    1392
Phe His Asn Phe Met Lys Glu Lys His Trp Gln Leu Asn Gly Leu Gln
    450                 455                 460

TTC CCA GCT GGA GTT CAT ATC ATG GTC ACT ATG AAT CAT ACT CAT CCT    1440
Phe Pro Ala Gly Val His Ile Met Val Thr Met Asn His Thr His Pro
```

465 470 475 480

GGA CTC GCT GAA GCT TTC GTC GCC GAT TGC AGA GCT GCA GTT GAG TTT 1488
Gly Leu Ala Glu Ala Phe Val Ala Asp Cys Arg Ala Ala Val Glu Phe
485 490 495

GTC AAA AGC CAC AAA CCA TCG GAA TCC GAC AAG ACA AGT GAA GCA GCC 1536
Val Lys Ser His Lys Pro Ser Glu Ser Asp Lys Thr Ser Glu Ala Ala
500 505 510

ATC TAC GGA CTT GCT CAA AGT ATT CCA GAC CGA TCG CTT GTT CAC GAG 1584
Ile Tyr Gly Leu Ala Gln Ser Ile Pro Asp Arg Ser Leu Val His Glu
515 520 525

TTT GCT CAC AGC TAT ATC GAT GCT GTT TAT GCT TTA ACA GAG 1626
Phe Ala His Ser Tyr Ile Asp Ala Val Tyr Ala Leu Thr Glu
530 535 540

TGA 1629

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Phe Ala Leu Glu Gln Tyr His Ser Ala Lys Asp Leu Leu Ile
1 5 10 15

Phe Glu Leu Arg Lys Phe Asn Pro Ile Val Leu Val Ser Ser Thr Ile
20 25 30

```
Val Ala Thr Tyr Val Leu Thr Asn Leu Arg His Met His Leu Asp Glu
         35                  40                  45

Met Gly Ile Arg Lys Arg Leu Ser Thr Trp Phe Phe Thr Thr Val Lys
     50                  55                  60

Arg Val Pro Phe Ile Arg Lys Met Ile Asp Lys Gln Leu Asn Glu Val
 65                  70                  75                  80

Lys Asp Glu Leu Glu Lys Ser Leu Arg Ile Val Asp Arg Ser Thr Glu
                85                  90                  95

Tyr Phe Thr Thr Ile Pro Ser His Ser Val Gly Arg Thr Glu Val Leu
            100                 105                 110

Arg Leu Ala Ala Ile Tyr Asp Asp Leu Glu Gly Pro Ala Phe Leu Glu
        115                 120                 125

Gly Arg Val Ser Gly Ala Val Phe Asn Arg Glu Asp Asp Lys Asp Glu
    130                 135                 140

Arg Glu Met Tyr Glu Glu Val Phe Gly Lys Phe Ala Trp Thr Asn Pro
145                 150                 155                 160

Leu Trp Pro Lys Leu Phe Pro Gly Val Arg Ile Met Glu Ala Glu Val
                165                 170                 175

Val Arg Met Cys Cys Asn Met Met Asn Gly Asp Ser Glu Thr Cys Gly
            180                 185                 190

Thr Met Ser Thr Gly Gly Ser Ile Ser Ile Leu Leu Ala Cys Leu Ala
        195                 200                 205

His Arg Asn Arg Leu Leu Lys Arg Gly Glu Lys Tyr Thr Glu Met Ile
    210                 215                 220
```

Val Pro Ser Ser Val His Ala Ala Phe Phe Lys Ala Ala Glu Cys Phe
225 230 235 240

Arg Ile Lys Val Arg Lys Ile Pro Val Asp Pro Val Thr Phe Lys Val
245 250 255

Asp Leu Val Lys Met Lys Ala Ala Ile Asn Lys Arg Thr Cys Met Leu
260 265 270

Val Gly Ser Ala Pro Asn Phe Pro Phe Gly Thr Val Asp Asp Ile Glu
275 280 285

Ala Ile Gly Gln Leu Gly Leu Glu Tyr Asp Ile Pro Val His Val Asp
290 295 300

Ala Cys Leu Gly Gly Phe Leu Leu Pro Phe Leu Glu Glu Asp Glu Ile
305 310 315 320

Arg Tyr Asp Phe Arg Val Pro Gly Val Ser Ser Ile Ser Ala Asp Ser
325 330 335

His Lys Tyr Gly Leu Ala Pro Lys Gly Ser Ser Val Val Leu Tyr Arg
340 345 350

Asn Lys Glu Leu Leu His Asn Gln Tyr Phe Cys Asp Ala Asp Trp Gln
355 360 365

Gly Gly Ile Tyr Ala Ser Ala Thr Met Glu Gly Ser Arg Ala Gly His
370 375 380

Asn Ile Ala Leu Cys Trp Ala Ala Met Leu Tyr His Ala Gln Glu Gly
385 390 395 400

Tyr Lys Ala Asn Ala Arg Lys Ile Val Asp Thr Thr Arg Lys Ile Arg
405 410 415

Asn Gly Leu Ser Asn Ile Lys Gly Ile Lys Leu Gln Gly Pro Ser Asp

420 425 430

Val Cys Ile Val Ser Trp Thr Thr Asn Asp Gly Val Glu Leu Tyr Arg
435 440 445

Phe His Asn Phe Met Lys Glu Lys His Trp Gln Leu Asn Gly Leu Gln
450 455 460

Phe Pro Ala Gly Val His Ile Met Val Thr Met Asn His Thr His Pro
465 470 475 480

Gly Leu Ala Glu Ala Phe Val Ala Asp Cys Arg Ala Ala Val Glu Phe
485 490 495

Val Lys Ser His Lys Pro Ser Glu Ser Asp Lys Thr Ser Glu Ala Ala
500 505 510

Ile Tyr Gly Leu Ala Gln Ser Ile Pro Asp Arg Ser Leu Val His Glu
515 520 525

Phe Ala His Ser Tyr Ile Asp Ala Val Tyr Ala Leu Thr Glu
530 535 540

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1770 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1767

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG AGT GGA GTA TCA AAT AAA ACA GTA TCA ATT AAT GGT TGG TAT GGC      48
Met Ser Gly Val Ser Asn Lys Thr Val Ser Ile Asn Gly Trp Tyr Gly
 1               5                  10                  15

ATG CCA ATT CAT TTA CTA AGG GAA GAA GGC GAC TTT GCC CAG TTT ATG      96
Met Pro Ile His Leu Leu Arg Glu Glu Gly Asp Phe Ala Gln Phe Met
            20                  25                  30

ATT CTA ACC ATC AAC GAA TTA AAA ATA GCC ATA CAT GGT TAC CTC AGA     144
Ile Leu Thr Ile Asn Glu Leu Lys Ile Ala Ile His Gly Tyr Leu Arg
        35                  40                  45

AAT ACC CCA TGG TAC AAC ATG TTG AAG GAT TAT TTG TTT GTG ATC TTT     192
Asn Thr Pro Trp Tyr Asn Met Leu Lys Asp Tyr Leu Phe Val Ile Phe
    50                  55                  60

TGT TAC AAG CTA ATA AGT AAT TTT TTT TAT CTG TTG AAA GTT TAT GGG     240
Cys Tyr Lys Leu Ile Ser Asn Phe Phe Tyr Leu Leu Lys Val Tyr Gly
65                  70                  75                  80

CCG GTG AGG TTA GCA GTG AGA ACA TAC GAG CAT AGT TCC AGA AGA TTG     288
Pro Val Arg Leu Ala Val Arg Thr Tyr Glu His Ser Ser Arg Arg Leu
                85                  90                  95

TTT CGT TGG TTA TTG GAC TCA CCA TTT TTG AGG GGT ACC GTA GAA AAG     336
Phe Arg Trp Leu Leu Asp Ser Pro Phe Leu Arg Gly Thr Val Glu Lys
            100                 105                 110

GAA GTC ACA AAG GTC AAA CAA TCG ATC GAA GAC GAA CTA ATT AGA TCG     384
Glu Val Thr Lys Val Lys Gln Ser Ile Glu Asp Glu Leu Ile Arg Ser
        115                 120                 125

GAC TCT CAG TTA ATG AAT TTC CCA CAG TTG CCA TCC AAT GGG ATA CCT     432
Asp Ser Gln Leu Met Asn Phe Pro Gln Leu Pro Ser Asn Gly Ile Pro
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    130                 135                 140

CAG GAT GAT GTT ATT GAA GAG CTA AAT AAA TTG AAC GAC TTG ATA CCA      480
Gln Asp Asp Val Ile Glu Glu Leu Asn Lys Leu Asn Asp Leu Ile Pro
145                 150                 155                 160

CAT ACC CAA TGG AAG GAA GGA AAG GTC TCT GGT GCC GTT TAC CAC GGT      528
His Thr Gln Trp Lys Glu Gly Lys Val Ser Gly Ala Val Tyr His Gly
                165                 170                 175

GGT GAT GAT TTG ATC CAC TTA CAA ACA ATC GCA TAC GAA AAA TAT TGC      576
Gly Asp Asp Leu Ile His Leu Gln Thr Ile Ala Tyr Glu Lys Tyr Cys
            180                 185                 190

GTT GCC AAT CAA TTA CAT CCC GAT GTC TTT CCT GCC GTA CGT AAA ATG      624
Val Ala Asn Gln Leu His Pro Asp Val Phe Pro Ala Val Arg Lys Met
        195                 200                 205

GAA TCC GAA GTG GTT TCT ATG GTT TTA AGA ATG TTT AAT GCC CCT TCT      672
Glu Ser Glu Val Val Ser Met Val Leu Arg Met Phe Asn Ala Pro Ser
    210                 215                 220

GAT ACA GGT TGT GGT ACC ACA ACT TCA GGT GGT ACA GAA TCC TTG CTT      720
Asp Thr Gly Cys Gly Thr Thr Thr Ser Gly Gly Thr Glu Ser Leu Leu
225                 230                 235                 240

TTA GCA TGT CTG AGC GCT AAA ATG TAT GCC CTT CAT CAT CGT GGA ATC      768
Leu Ala Cys Leu Ser Ala Lys Met Tyr Ala Leu His His Arg Gly Ile
                245                 250                 255

ACC GAA CCA GAA ATA ATT GCT CCC GTA ACT GCA CAT GCT GGG TTT GAC      816
Thr Glu Pro Glu Ile Ile Ala Pro Val Thr Ala His Ala Gly Phe Asp
            260                 265                 270

AAA GCT GCT TAT TAC TTT GGC ATG AAG CTA CGC CAC GTG GAG CTA GAT      864
Lys Ala Ala Tyr Tyr Phe Gly Met Lys Leu Arg His Val Glu Leu Asp
        275                 280                 285
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CCA ACG ACA TAT CAA GTG GAC CTG GGA AAA GTG AAA AAA TTC ATC AAT 912
Pro Thr Thr Tyr Gln Val Asp Leu Gly Lys Val Lys Lys Phe Ile Asn
290 295 300

AAG AAC ACA ATT TTA CTG GTC GGT TCC GCT CCA AAC TTT CCT CAT GGT 960
Lys Asn Thr Ile Leu Leu Val Gly Ser Ala Pro Asn Phe Pro His Gly
305 310 315 320

ATT GCC GAT GAT ATT GAA GGA TTG GGT AAA ATA GCA CAA AAA TAT AAA 1008
Ile Ala Asp Asp Ile Glu Gly Leu Gly Lys Ile Ala Gln Lys Tyr Lys
325 330 335

CTT CCT TTA CAC GTC GAC AGT TGT CTA GGT TCC TTT ATT GTT TCA TTT 1056
Leu Pro Leu His Val Asp Ser Cys Leu Gly Ser Phe Ile Val Ser Phe
340 345 350

ATG GAA AAG GCT GGT TAC AAA AAT CTG CCA TTA CTT GAC TTT AGA GTC 1104
Met Glu Lys Ala Gly Tyr Lys Asn Leu Pro Leu Leu Asp Phe Arg Val
355 360 365

CCG GGA GTC ACC TCA ATA TCA TGT GAC ACT CAT AAA TAT GGA TTT GCA 1152
Pro Gly Val Thr Ser Ile Ser Cys Asp Thr His Lys Tyr Gly Phe Ala
370 375 380

CCA AAA GGC TCG TCA GTT ATA ATG TAT AGA AAC AGC GAC TTA CGA ATG 1200
Pro Lys Gly Ser Ser Val Ile Met Tyr Arg Asn Ser Asp Leu Arg Met
385 390 395 400

CAT CAG TAT TAC GTA AAT CCT GCT TGG ACT GGC GGG TTA TAT GGC TCT 1248
His Gln Tyr Tyr Val Asn Pro Ala Trp Thr Gly Gly Leu Tyr Gly Ser
405 410 415

CCT ACA TTA GCA GGG TCC AGG CCT GGT GCT ATT GTC GTA GGT TGT TGG 1296
Pro Thr Leu Ala Gly Ser Arg Pro Gly Ala Ile Val Val Gly Cys Trp
420 425 430

```
GCC ACT ATG GTC AAC ATG GGT GAA AAT GGG TAC ATT GAG TCG TGC CAA      1344
Ala Thr Met Val Asn Met Gly Glu Asn Gly Tyr Ile Glu Ser Cys Gln
        435                 440                 445

GAA ATA GTC GGT GCA GCA ATG AAG TTT AAA AAA TAC ATC CAG GAA AAC      1392
Glu Ile Val Gly Ala Ala Met Lys Phe Lys Lys Tyr Ile Gln Glu Asn
    450                 455                 460

ATT CCA GAC CTG AAT ATA ATG GGC AAC CCT AGA TAT TCA GTC ATT TCA      1440
Ile Pro Asp Leu Asn Ile Met Gly Asn Pro Arg Tyr Ser Val Ile Ser
465                 470                 475                 480

TTT TCT TCA AAG ACC TTG AAC ATA CAC GAA CTA TCT GAC AGG TTG TCC      1488
Phe Ser Ser Lys Thr Leu Asn Ile His Glu Leu Ser Asp Arg Leu Ser
                485                 490                 495

AAG AAA GGC TGG CAT TTC AAT GCC CTA CAA AAG CCG GTT GCA CTA CAC      1536
Lys Lys Gly Trp His Phe Asn Ala Leu Gln Lys Pro Val Ala Leu His
            500                 505                 510

ATG GCC TTC ACG AGA TTG AGC GCT CAT GTT GTG GAT GAG ATC TGC GAC      1584
Met Ala Phe Thr Arg Leu Ser Ala His Val Val Asp Glu Ile Cys Asp
        515                 520                 525

ATT TTA CGT ACT ACC GTG CAA GAG TTG AAG AGC GAA TCA AAT TCT AAA      1632
Ile Leu Arg Thr Thr Val Gln Glu Leu Lys Ser Glu Ser Asn Ser Lys
    530                 535                 540

CCA TCC CCA GAC GGA ACT AGC GCT CTA TAT GGT GTC GCC GGG AGC GTT      1680
Pro Ser Pro Asp Gly Thr Ser Ala Leu Tyr Gly Val Ala Gly Ser Val
545                 550                 555                 560

AAA ACT GCT GGC GTT GCA GAC AAA TTG ATT GTG GGA TTC CTA GAC GCA      1728
Lys Thr Ala Gly Val Ala Asp Lys Leu Ile Val Gly Phe Leu Asp Ala
                565                 570                 575

TTA TAC AAG TTG GGT CCA GGA GAG GAT ACC GCC ACC AAG TAG              1770
```

Leu Tyr Lys Leu Gly Pro Gly Glu Asp Thr Ala Thr Lys
580 585

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 589 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Gly Val Ser Asn Lys Thr Val Ser Ile Asn Gly Trp Tyr Gly
1 5 10 15

Met Pro Ile His Leu Leu Arg Glu Glu Gly Asp Phe Ala Gln Phe Met
20 25 30

Ile Leu Thr Ile Asn Glu Leu Lys Ile Ala Ile His Gly Tyr Leu Arg
35 40 45

Asn Thr Pro Trp Tyr Asn Met Leu Lys Asp Tyr Leu Phe Val Ile Phe
50 55 60

Cys Tyr Lys Leu Ile Ser Asn Phe Phe Tyr Leu Leu Lys Val Tyr Gly
65 70 75 80

Pro Val Arg Leu Ala Val Arg Thr Tyr Glu His Ser Ser Arg Arg Leu
85 90 95

Phe Arg Trp Leu Leu Asp Ser Pro Phe Leu Arg Gly Thr Val Glu Lys
100 105 110

Glu Val Thr Lys Val Lys Gln Ser Ile Glu Asp Glu Leu Ile Arg Ser

115 120 125

Asp Ser Gln Leu Met Asn Phe Pro Gln Leu Pro Ser Asn Gly Ile Pro
130 135 140

Gln Asp Asp Val Ile Glu Glu Leu Asn Lys Leu Asn Asp Leu Ile Pro
145 150 155 160

His Thr Gln Trp Lys Glu Gly Lys Val Ser Gly Ala Val Tyr His Gly
165 170 175

Gly Asp Asp Leu Ile His Leu Gln Thr Ile Ala Tyr Glu Lys Tyr Cys
180 185 190

Val Ala Asn Gln Leu His Pro Asp Val Phe Pro Ala Val Arg Lys Met
195 200 205

Glu Ser Glu Val Val Ser Met Val Leu Arg Met Phe Asn Ala Pro Ser
210 215 220

Asp Thr Gly Cys Gly Thr Thr Thr Ser Gly Gly Thr Glu Ser Leu Leu
225 230 235 240

Leu Ala Cys Leu Ser Ala Lys Met Tyr Ala Leu His His Arg Gly Ile
245 250 255

Thr Glu Pro Glu Ile Ile Ala Pro Val Thr Ala His Ala Gly Phe Asp
260 265 270

Lys Ala Ala Tyr Tyr Phe Gly Met Lys Leu Arg His Val Glu Leu Asp
275 280 285

Pro Thr Thr Tyr Gln Val Asp Leu Gly Lys Val Lys Lys Phe Ile Asn
290 295 300

Lys Asn Thr Ile Leu Leu Val Gly Ser Ala Pro Asn Phe Pro His Gly
305 310 315 320

Ile Ala Asp Asp Ile Glu Gly Leu Gly Lys Ile Ala Gln Lys Tyr Lys
325 330 335

Leu Pro Leu His Val Asp Ser Cys Leu Gly Ser Phe Ile Val Ser Phe
340 345 350

Met Glu Lys Ala Gly Tyr Lys Asn Leu Pro Leu Leu Asp Phe Arg Val
355 360 365

Pro Gly Val Thr Ser Ile Ser Cys Asp Thr His Lys Tyr Gly Phe Ala
370 375 380

Pro Lys Gly Ser Ser Val Ile Met Tyr Arg Asn Ser Asp Leu Arg Met
385 390 395 400

His Gln Tyr Tyr Val Asn Pro Ala Trp Thr Gly Gly Leu Tyr Gly Ser
405 410 415

Pro Thr Leu Ala Gly Ser Arg Pro Gly Ala Ile Val Val Gly Cys Trp
420 425 430

Ala Thr Met Val Asn Met Gly Glu Asn Gly Tyr Ile Glu Ser Cys Gln
435 440 445

Glu Ile Val Gly Ala Ala Met Lys Phe Lys Lys Tyr Ile Gln Glu Asn
450 455 460

Ile Pro Asp Leu Asn Ile Met Gly Asn Pro Arg Tyr Ser Val Ile Ser
465 470 475 480

Phe Ser Ser Lys Thr Leu Asn Ile His Glu Leu Ser Asp Arg Leu Ser
485 490 495

Lys Lys Gly Trp His Phe Asn Ala Leu Gln Lys Pro Val Ala Leu His
500 505 510

Met Ala Phe Thr Arg Leu Ser Ala His Val Val Asp Glu Ile Cys Asp
515 520 525

Ile Leu Arg Thr Thr Val Gln Glu Leu Lys Ser Glu Ser Asn Ser Lys
530 535 540

Pro Ser Pro Asp Gly Thr Ser Ala Leu Tyr Gly Val Ala Gly Ser Val
545 550 555 560

Lys Thr Ala Gly Val Ala Asp Lys Leu Ile Val Gly Phe Leu Asp Ala
565 570 575

Leu Tyr Lys Leu Gly Pro Gly Glu Asp Thr Ala Thr Lys
580 585

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1467 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1464

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG CCT AGC ACA GAC CTT CTG ATG TTG AAG GCC TTT GAG CCC TAC TTA 48
Met Pro Ser Thr Asp Leu Leu Met Leu Lys Ala Phe Glu Pro Tyr Leu
1 5 10 15

GAG ATT TTG GAA GTA TAC TCC ACA AAA GCC AAG AAT TAT GTA AAT GGA 96

Glu Ile Leu Glu Val Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
20 25 30

CAT TGC ACC AAG TAT GAG CCC TGG CAG CTA ATT GCA TGG AGT GTC GTG 144
His Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Val
35 40 45

TGG ACC CTG CTG ATA GTC TGG GGA TAT GAG TTT GTC TTC CAG CCA GAG 192
Trp Thr Leu Leu Ile Val Trp Gly Tyr Glu Phe Val Phe Gln Pro Glu
50 55 60

AGT TTA TGG TCA AGG TTT AAA AAG AAA TGT TTT AAG CTC ACC AGG AAG 240
Ser Leu Trp Ser Arg Phe Lys Lys Lys Cys Phe Lys Leu Thr Arg Lys
65 70 75 80

ATG CCC ATT ATT GGT CGT AAG ATT CAA GAC AAG TTG AAC AAG ACC AAG 288
Met Pro Ile Ile Gly Arg Lys Ile Gln Asp Lys Leu Asn Lys Thr Lys
85 90 95

GAT GAT ATT AGC AAG AAC ATG TCA TTC CTG AAA GTG GAC AAA GAG TAT 336
Asp Asp Ile Ser Lys Asn Met Ser Phe Leu Lys Val Asp Lys Glu Tyr
100 105 110

GTG AAA GCT TTA CCC TCC CAG GGT CTG AGC TCA TCT GCT GTT TTG GAG 384
Val Lys Ala Leu Pro Ser Gln Gly Leu Ser Ser Ser Ala Val Leu Glu
115 120 125

AAA CTT AAG GAG TAC AGC TCT ATG GAC GCC TTC TGG CAA GAG GGG AGA 432
Lys Leu Lys Glu Tyr Ser Ser Met Asp Ala Phe Trp Gln Glu Gly Arg
130 135 140

GCC TCT GGA ACA GTG TAC AGT GGG GAG GAG AAG CTC ACT GAG CTC CTT 480
Ala Ser Gly Thr Val Tyr Ser Gly Glu Glu Lys Leu Thr Glu Leu Leu
145 150 155 160

GTG AAG GCT TAT GGA GAT TTT GCA TGG AGT AAC CCC CTG CAT CCA GAT 528
Val Lys Ala Tyr Gly Asp Phe Ala Trp Ser Asn Pro Leu His Pro Asp

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                165                 170                 175

ATC TTC CCA GGA CTA CGC AAG ATA GAG GCA GAA ATT GTG AGG ATA GCT      576
Ile Phe Pro Gly Leu Arg Lys Ile Glu Ala Glu Ile Val Arg Ile Ala
            180                 185                 190

TGT TCC CTG TTC AAT GGG GGA CCA GAT TCG TGT GGA TGT GTG ACT TCT      624
Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
        195                 200                 205

GGG GGA ACA GAA AGC ATA CTC ATG GCC TGC AAA GCA TGT CGG GAT CTG      672
Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Cys Arg Asp Leu
    210                 215                 220

GCC TTT GAG AAG GGG ATC AAA ACT CCA GAA ATT GTG GCT CCC CAA AGT      720
Ala Phe Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Gln Ser
225                 230                 235                 240

GCC CAT GCT GCA TTT AAC AAA GCA GCC AGT TAC TTT GGG ATG AAG ATT      768
Ala His Ala Ala Phe Asn Lys Ala Ala Ser Tyr Phe Gly Met Lys Ile
                245                 250                 255

GTG CGG GTC CCA TTG ACG AAG ATG ATG GAG GTG GAT GTG AGG GCA ATG      816
Val Arg Val Pro Leu Thr Lys Met Met Glu Val Asp Val Arg Ala Met
            260                 265                 270

AGA AGA GCT ATC TCC AGG AAC ACT GCC ATG CTC GTC TGT TCT ACC CCA      864
Arg Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
        275                 280                 285

CAG TTT CCT CAT GGT GTA ATA GAT CCT GTC CCT GAA GTG GCC AAG CTG      912
Gln Phe Pro His Gly Val Ile Asp Pro Val Pro Glu Val Ala Lys Leu
    290                 295                 300

GCT GTC AAA TAC AAA ATA CCC CTT CAT GTC GAC GCT TGT CTG GGA GGC      960
Ala Val Lys Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305                 310                 315                 320
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TTC CTC ATC GTC TTT ATG GAG AAA GCA GGA TAC CCA CTG GAG CAC CCA 1008
Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu His Pro
325 330 335

TTT GAT TTC CGG GTG AAA GGT GTA ACC AGC ATT TCA GCT GAC ACC CAT 1056
Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
340 345 350

AAG CTG GAA AAT ATC AAA GGC ATC TTT GTT TTT GGG AAT CCC CAA TTG 1104
Lys Leu Glu Asn Ile Lys Gly Ile Phe Val Phe Gly Asn Pro Gln Leu
355 360 365

TCA CTC ATT GCT CTG GGA TCC CGT GAT TTT GAC ATC TAC CGA CTA TCA 1152
Ser Leu Ile Ala Leu Gly Ser Arg Asp Phe Asp Ile Tyr Arg Leu Ser
370 375 380

AAC CTG ATG ACT GCT AAG GGG TGG AAC TTG AAC CAG TTG CAG TTC CCA 1200
Asn Leu Met Thr Ala Lys Gly Trp Asn Leu Asn Gln Leu Gln Phe Pro
385 390 395 400

CCC AGT ATT CAT TTC TGC ATC ACA TTA CTA CAC GCC CGG AAA CGA GTA 1248
Pro Ser Ile His Phe Cys Ile Thr Leu Leu His Ala Arg Lys Arg Val
405 410 415

GCT ATA CAA TTC CTA AAG GAC ATT CGA GAA TCT GTC ACT CAA ATC ATG 1296
Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
420 425 430

AAG AAT CCT AAA GCG AAG ACC ACA GGA ATG GGT GCC ATC TAT GCC ATG 1344
Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Ala Met
435 440 445

GCC CAG ACA ACT GTT GAC AGG AAT ATG GTT GCA GAA TTG TCC TCA GTC 1392
Ala Gln Thr Thr Val Asp Arg Asn Met Val Ala Glu Leu Ser Ser Val
450 455 460

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TTC TTG GAC AGC TTG TAC AGC ACC GAC ACT GTC ACC CAG GGC AGC CAG      1440
Phe Leu Asp Ser Leu Tyr Ser Thr Asp Thr Val Thr Gln Gly Ser Gln
465                 470                 475                 480

ATG AAT GGT TCT CCA AAA CCC CAC TGA                              1467
Met Asn Gly Ser Pro Lys Pro His
                485
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 488 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Pro Ser Thr Asp Leu Leu Met Leu Lys Ala Phe Glu Pro Tyr Leu
 1               5                  10                  15

Glu Ile Leu Glu Val Tyr Ser Thr Lys Ala Lys Asn Tyr Val Asn Gly
            20                  25                  30

His Cys Thr Lys Tyr Glu Pro Trp Gln Leu Ile Ala Trp Ser Val Val
        35                  40                  45

Trp Thr Leu Leu Ile Val Trp Gly Tyr Glu Phe Val Phe Gln Pro Glu
    50                  55                  60

Ser Leu Trp Ser Arg Phe Lys Lys Lys Cys Phe Lys Leu Thr Arg Lys
65                  70                  75                  80

Met Pro Ile Ile Gly Arg Lys Ile Gln Asp Lys Leu Asn Lys Thr Lys
                85                  90                  95
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Asp Asp Ile Ser Lys Asn Met Ser Phe Leu Lys Val Asp Lys Glu Tyr
100 105 110

Val Lys Ala Leu Pro Ser Gln Gly Leu Ser Ser Ser Ala Val Leu Glu
115 120 125

Lys Leu Lys Glu Tyr Ser Ser Met Asp Ala Phe Trp Gln Glu Gly Arg
130 135 140

Ala Ser Gly Thr Val Tyr Ser Gly Glu Glu Lys Leu Thr Glu Leu Leu
145 150 155 160

Val Lys Ala Tyr Gly Asp Phe Ala Trp Ser Asn Pro Leu His Pro Asp
165 170 175

Ile Phe Pro Gly Leu Arg Lys Ile Glu Ala Glu Ile Val Arg Ile Ala
180 185 190

Cys Ser Leu Phe Asn Gly Gly Pro Asp Ser Cys Gly Cys Val Thr Ser
195 200 205

Gly Gly Thr Glu Ser Ile Leu Met Ala Cys Lys Ala Cys Arg Asp Leu
210 215 220

Ala Phe Glu Lys Gly Ile Lys Thr Pro Glu Ile Val Ala Pro Gln Ser
225 230 235 240

Ala His Ala Ala Phe Asn Lys Ala Ala Ser Tyr Phe Gly Met Lys Ile
245 250 255

Val Arg Val Pro Leu Thr Lys Met Met Glu Val Asp Val Arg Ala Met
260 265 270

Arg Arg Ala Ile Ser Arg Asn Thr Ala Met Leu Val Cys Ser Thr Pro
275 280 285

Gln Phe Pro His Gly Val Ile Asp Pro Val Pro Glu Val Ala Lys Leu

290 295 300

Ala Val Lys Tyr Lys Ile Pro Leu His Val Asp Ala Cys Leu Gly Gly
305 310 315 320

Phe Leu Ile Val Phe Met Glu Lys Ala Gly Tyr Pro Leu Glu His Pro
325 330 335

Phe Asp Phe Arg Val Lys Gly Val Thr Ser Ile Ser Ala Asp Thr His
340 345 350

Lys Leu Glu Asn Ile Lys Gly Ile Phe Val Phe Gly Asn Pro Gln Leu
355 360 365

Ser Leu Ile Ala Leu Gly Ser Arg Asp Phe Asp Ile Tyr Arg Leu Ser
370 375 380

Asn Leu Met Thr Ala Lys Gly Trp Asn Leu Asn Gln Leu Gln Phe Pro
385 390 395 400

Pro Ser Ile His Phe Cys Ile Thr Leu Leu His Ala Arg Lys Arg Val
405 410 415

Ala Ile Gln Phe Leu Lys Asp Ile Arg Glu Ser Val Thr Gln Ile Met
420 425 430

Lys Asn Pro Lys Ala Lys Thr Thr Gly Met Gly Ala Ile Tyr Ala Met
435 440 445

Ala Gln Thr Thr Val Asp Arg Asn Met Val Ala Glu Leu Ser Ser Val
450 455 460

Phe Leu Asp Ser Leu Tyr Ser Thr Asp Thr Val Thr Gln Gly Ser Gln
465 470 475 480

Met Asn Gly Ser Pro Lys Pro His
485

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(71) Applicant: CHILDREN'S HOSPITAL OAKLAND RESEARCH INSTITUTE [US/US]; 747 Fifty Second Street, Oakland, CA 96409–1809 (US).

(72) Inventors: SABA, Julie, D.; Apartment 7317, 6401 Bay Street, Emeryville, CA 95608 (US). ZHOU, Jianhui; 246 Bearritz Court, Redwood City, CA 94065 (US).

(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

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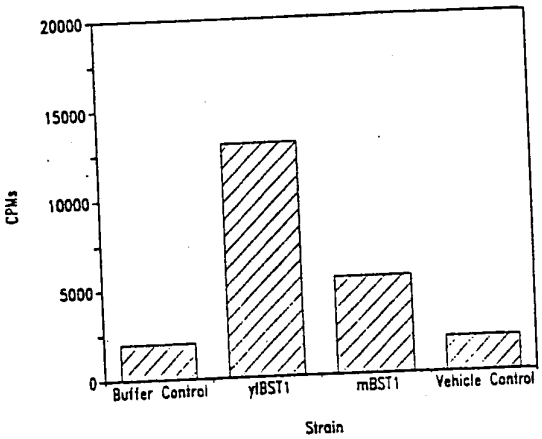
(54) Title: SPHINGOSINE–1–PHOSPHATE LYASE POLYPEPTIDES, POLYNUCLEOTIDES AND MODULATING AGENTS AND METHODS OF USE THEREFOR

(57) Abstract

Compositions, methods and kits for diagnosing and treating cancer are provided. Therapeutic compositions may comprise agents that modulate the expression or activity of a sphingosine–1–phosphate lyase (SPL). Such compositions may be administered to a mammal afflicted with cancer. Diagnostic methods and kits may employ an agent suitable for detecting alterations in endogenous SPL. Such methods and kits may be used to detect the presence of a cancer or to evaluate the prognosis of a known disease. SPL polypeptides, polynucleotides and antibodies are also provided.



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EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/20365

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/60 C12N5/10 C12N1/21 C12N9/88 C12Q1/68
C07K16/40 G01N33/50 G01N33/68 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN VELDHOVEN P AND MANNAERTS G: "Sphingosine-Phosphate Lyase" ADV. LIPID RES., vol. 26, 1993, pages 69-98, XP002097298	1-12,19, 20,23, 44-47
Y	see section III in particular	13,14, 17,24, 27-29, 32,35,36

Y	WO 93 19760 A (BIOMEMBRANE INST) 14 October 1993 see abstract	13,14, 17,24, 27,29,32
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 1999	08/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No
Y	SPIEGEL S ET AL: "Sphingosine-1-phosphate. a novel second messenger involved in cell growth regulation and signal transduction. affects growth and invasiveness of human breast cancer cells" BREAST CANCER RES TREAT, vol. 31, no. 2-3, 1994, pages 337-348, XP002097299 see abstract	28.35.36
X	US 5 430 169 A (BOUMENDJEL AHCENE ET AL) 4 July 1995	19
Y	see abstract	13,17
X	DATABASE EMEST12 E.M.B.L. Databases Accession Number: T86263, 30 March 1995 HILLIER L ET AL: "Homo sapiens cDNA clone 114967" XP002097302 97.2% identity in 316bp overlap see abstract	1,6
X	DATABASE EMEST17 E.M.B.L. Databases Accession Number: AA338781, 18 April 1997 ADAMS M ET AL: "EST44070 Fetal brain I Homo sapiens cDNA 5' end" XP002097303 97.1% identity in 243bp overlap see abstract	1,6
X	DATABASE EMEST20 E.M.B.L. Databases Accession Number: AA107456, 6 November 1996 MARRA M ET AL: "The WashU-HHMI Mouse EST Project" XP002097304 100% identity in 427bp overlap see abstract	1,6
X	DATABASE EMEST1 E.M.B.L. Databases Accession Number: AA589412, 18 September 1997 MARRA M ET AL: "The WashU-HHMI Mouse EST Project" XP002097305 100% identity in 379bp overlap see abstract	1,6

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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 98/20365

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SABA J ET AL: "The BST1 gene of Saccharomyces cerevisiae is the sphingosine-1-phosphate lyase" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 272, no. 42, 17 October 1997, pages 26087-26090, XP002097300 see the whole document ---	1-9
P,X	ZHOU J AND SABA J: "Identification of the First Mammalian Sphingosine Phosphate Lyase Gene and Its Functional Expression in Yeast" BIOCHEM. BIOPHYS. RES. COMMUN., vol. 242, 1998, pages 502-507, XP002097301 see the whole document ---	1-9
A	QIE L ET AL: "Identification of a Saccharomyces gene, LCB3, necessary for incorporation of exogenous long chain bases into sphingolipids" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 272, no. 26, 27 June 1997, pages 16110-16117, XP002097443 see page 16110, column 2, line 31 - line 33 -----	

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 98/ 20365

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 19 through 36, as far as in vivo method are concerned are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/US 98/20365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9319760 A	14-10-1993	US 5260288 A	09-11-1993
		CA 2130992 A	14-10-1993
		EP 0671917 A	20-09-1995
		JP 8500816 T	30-01-1996
		US 5391800 A	21-02-1995
		US 5663404 A	02-09-1997
		US 5877167 A	02-03-1999
US 5430169 A	04-07-1995	AU 1874295 A	29-08-1995
		WO 9521848 A	17-08-1995